LABORATORY GUIDE

FOR THE STUDY OF

THE FROG

AN INTRODUCTION TO ANATOMY, HISTOLOGY AND PHYSIOLOGY

BY

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PREFACE.

For the introductory study of the structure and physiology of a typical vertebrate, there is no form better adapted than the common frog. The present course is based largely on the well-known works of Huxley, Marshall and Holmes; the author's task has been mainly that of simplification and adaptation, primarily to meet the needs of his own students. These directions are the outcome of eight years' experience in teaching the biology of the frog in the University of Michigan, Syracuse University, the University of Wisconsin and the Michigan State Normal College; the author is naturally much indebted to his former teachers and associates, particularly to Prof. S. J. Holmes.

As a general rule, the order of topics in the text should be followed, since it is planned to give a distinct picture of each organ system in its relation to the whole, with the greatest economy of time and material. For pedagogical reasons an exception should be made in the case of certain histological topics: it seems best to begin the microscopical work with the study of some simple tissues, such as the stratum corneum, cartilage, and perhaps also blood, connective tissue and unstriated muscle fiber, before attempting the study of cross-sections of such complex structures as the alimentary canal and the kidney. By following the sequence of topics in this manual, all the study of gross anatomy, excepting the work on the circulatory system and the skeleton, may be done with a single specimen.

In connection with the laboratory work the student should read the corresponding portions of Holmes' Biology of the Frog. As a rule the reading on a given topic should follow, rather than precede, the laboratory study of that topic.

In case time is limited, the work may be shortened by a judicious omission of topics. The chapters on the eye and the ear, the thyroid glands, and even the skeleton and the muscles, may be omitted without serious detriment to the remainder of the work. In case material is limited, an entire frog may be saved for each student by omitting the special dissection of the venous system.

A first-hand knowledge of biological material, and training of the powers of observation and interpretation of biological phenomena, are assumed to be the primary aims of laboratory work in this field. In this guide these ends are sought by a combination of the verification method of Huxley with the investigation or problem-solving method of Agassiz, in proportions suited to the needs of students with little or no previous experience in biological laboratory work. In using the verification method it is believed that more valuable results will be obtained if descriptions, rather than drawings or diagrams, are used as a guide for study; drawings should be made by the student himself, as a record of what he has actually observed. In the solution of problems involving more than a single observation, the motto of the student should be "divide and conquer"; by focussing the attention upon one feature at a time, the various elements of a complex structure or process are gradually revealed. When properly carried out, such a laboratory course should represent not only an important accessory to the class-room instruction in biological principles, but a training which in itself is of the highest value to the general student as well as to the future biologist.

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PRELIMINARY DIRECTIONS.

I. Directions for Preparing a Laboratory Notebook in Zoology.

A. **Materials.**—Each student should provide:

1. Glencoe covers fastened with a shoe string, to be used for filing finished, corrected notes and drawings.

2. One-half pound note paper, ruled on both sides, to fit Glencoe covers.

3. A dozen sheets Buckeye Linen Ledger drawing paper to fit Glencoe covers.

4. Two sheets thin cardboard (either manilla or "mounting board"), to fit Glencoe covers.

5. Four manilla envelopes, to be used for storing the blank paper and uncorrected work. The student's name and seat number should be placed in the upper right-hand corner; the class and hour in the upper left-hand corner. The particular use of the envelope should be recorded in its center, thus: "Blank Note Paper"; "Blank Drawing Paper"; "Hand-in Envelope" (two of these can be used). On the last mentioned envelope the student's name should appear on the flap, also.

Items 1, 2, 3, 4 and 5 may be obtained from the dealers by asking for a "Zoölogy Note Book."

6. A 4H Kohinoor drawing pencil. A 5H or a 6H Kohinoor drawing pencil in addition is desirable, but is not absolutely necessary.

7. Lead eraser with beveled edge.

8. Pen, ink and blotter.

All the above should be kept in the laboratory and not be taken away for use elsewhere, except by special permission.

B. **Note Writing.**—1. The laboratory notebook is to be used only for laboratory work; lecture notes and information obtained by reading should not be included. This notebook is intended to represent so far as possible the results of a first hand study of nature and should record only direct observations actually made by the student and his inferences from these observations.

2. The laboratory notes will usually consist only of explicit and complete answers to questions asked or problems stated in the laboratory directions. The answer to a question should take the form of a complete statement; that is, in reading the answer one

should not be compelled to refer to the directions to find out what question is being answered. It is not required that all the observations called for in the laboratory directions be recorded, since this would involve too much repetition of statements already made in the directions.

3. In all laboratory notes pay particular attention to topical headings and to paragraphing. The paragraph should possess strict unity of subject matter.

4. Notes should be written in ink and only one side of the paper should be used; the writing should appear on the *right-hand page* of the open book after the paper is filed in the covers. The other side of the sheet may be used for the explanation of drawings (see below).

5. The notes should be written in permanent form at the time the observations are made. The pages should be numbered consecutively, using Arabic numerals.

C. **Drawings.**—1. Make the drawings of sufficient size to show all the details clearly. Do not crowd the drawings; a wide margin should be left on all sides.

2. The drawings are mainly outlines, and the lines should be sharp, clear and even—not broad, soft or irregular in width and distinctness. See that the pencils are kept sharp. In making outlines be careful that the ends of the lines meet accurately, not overlapping or crossing. Never leave two lines where only one is intended. Leave no gaps in outlines, but draw even small granules with a complete and perfect contour. The drawings are usually semi-diagrammatic, emphasizing certain features to be made clear to the exclusion of others; this may necessitate arranging the parts in a somewhat unnatural order.

3. Care should be taken to represent the proportions accurately. It is advisable to outline the drawings very faintly at first, so that the lines may be readily erased if necessary. In doing this use a very sharp pencil, preferably a hard one (5H). After the correct form is secured, all lines should be made sharp and clear.

If the object you are drawing is bilaterally symmetrical, draw a faint line down the middle of the paper to represent the axis of bilateral symmetry; this line will aid in keeping the two halves alike, and may afterwards be erased.

4. Shading should be used only when necessary to differentiate parts. The best method of shading is usually by stippling—that is, by the use of small *round* dots. Other methods of shading should be used only by advice of the instructor.

5. The drawings should be placed on the drawing paper in such a way that when placed in the notebook covers they will fall on the *right-hand* page of the open book. Only one side of the drawing paper should be used. At the top of each plate, midway between the two upper corners, write the name of the animal described. Each plate should bear a number in Roman numerals in the extreme upper right-hand corner (*e. g.*, Plate I, Plate II, etc.), and the student's name in the extreme upper left-hand corner. The drawings of each page or plate should be numbered with Arabic numerals beginning with 1, *e. g.*, Fig. 1, Fig. 2, etc.; this number should be placed below the drawing. Every part of each figure should be named by drawing a fine, straight line from the part to the exterior nearest the point indicated and placing at its end an appropriate abbreviation in an upright position. The guiding lines are best made with the aid of a ruler; they may radiate out in all directions from the figure or be arranged in a parallel manner to suit the situation. All labeling should be done with a lead pencil; ink should not appear on the plate.

6. Reference sheets of note paper, written in ink, should face each plate, bearing the words "Explanation of Plate" followed by the plate number in Roman numerals, on the line at the top of the page. On the reference sheet each figure should have a descriptive or explanatory title, full but concisely stated, giving the object of the drawing, condition of the material, position and point of view, enlargement, etc. At the close of the list of titles give an alphabetical list of the abbreviations used, with their meaning.

7. If any of the above points are not clear to you, consult a sample notebook which will be provided.

D. **Corrections.**—1. At intervals throughout the term's work you will be directed to place all drawings, reference sheets and notes of whatever kind you wish criticized, in one of the "Hand-in Envelopes," in the proper order, the first page facing the back of the envelope, and submit them to the instructor for criticism.

2. Criticisms of notes are not to be erased. Criticisms of drawings made on the drawings themselves may be erased. Criticisms may take the form of a reference to a section and paragraph of these directions.

3. Unsatisfactory work must be revised. Corrections of notes may be made on the same sheet, if there is room, or on additional sheets inserted for the purpose.

4. After corrections are made the work is all to be filed in the covers. At the close of the course the entire notebook is to be handed in for final inspection.

II. Individual Equipment Furnished by the Laboratory.

The student should check up the following equipment and report promptly any deficiencies that may occur at any time.

Compound microscope. Use the microscope whose number corresponds to the number of your place at the table.

Dissecting stand; dissecting lens; metallic plate, white on one side, black on the other.

Instrument box containing the following: **Large scissors, small scissors, large forceps, small forceps, scalpel, seeker, probe, blowpipe, two dissecting needles** mounted in handles, **bristle** with waxed tip.

Pipette (medicine dropper) with black bulb. This is to be used for washing out delicate dissections. The pipettes with white bulbs which may be found about the laboratory are for use with living material and should be carefully guarded from contact with poisons.

Emery paper (to be used for sharpening pencils) mounted on a wooden block.

Millimeter rule.

Booklet on the use and care of the microscope.

Lens paper. To avoid dust, this should be kept between the leaves of the booklet. When you need more, call for it at the supply table.

Absorbent paper. When you need more, call for it at the supply table.

Microscopical object slides and cover slips should not be kept in the lockers. They may be obtained from the supply table, to which they should be returned after use.

III. General Directions for Dissecting.

1. Keep all necessary instruments on the table before you where they can be found without loss of time.

2. If the specimen is being preserved in formalin always wash it thoroughly under the tap before beginning work.

3. The specimen should ordinarily be kept in a dissecting tray

while being dissected, but in the case of an animal as large as a frog it is seldom advisable to pin the specimen to the tray; much is gained by leaving the specimen free so that it can be turned over as occasion demands, or held in the hand in such a way as to expose the parts being studied. If it is found desirable to pin the specimen to the tray, see that the pins do not injure any important organs. Delicate dissections should be made under water, which supports the parts and gives better optical results.

4. Dissection consists of separating and exposing the parts with as little injury to them as possible. Only necessary cutting should be done; the general rule for dissecting is to *cut nothing unless you know what it is and why you cut it.* In dissecting it is often necessary to remove the connective tissue which binds the other parts together. The instruments in most constant use should be forceps, needles and seeker, rather than scalpel and scissors. When an incision is necessary, make it as clean as possible—avoid scrappy or haphazard work. Do not *peck* the specimen.

In cleaning blood-vessels or nerves always dissect along them and not across them, and avoid laying hold of them with the forceps. When separating muscles dissect along their fibers and not across them.

5. Keep the parts being dissected clean: free from debris, mucus, etc. Delicate dissections may be washed with a gentle stream of water from a pipette.

6. Scalpels should be kept sharp. A whetstone is provided and students will be instructed how to use it.

7. Instruments should be used only for the purposes intended.

8. The student should provide a towel for individual use.

9. At the close of the laboratory period return your specimen to the preserving fluid; rinse out your tray and place it on edge in the rack to dry; clean your instruments, wipe them dry with a soft cloth or towel and return them to your locker.

IV. Preliminary Exercise on the Use of the Compound Microscope.

A. With the aid of an instructor identify the following parts of the microscope: Stage, diaphragm, mirror, tube, ocular or eye-piece, objective, coarse adjustment, fine adjustment.

Place an object, mounted on a slide and covered with a cover glass, in the center of the stage. Adjust the *concave* mirror and the diaphragm so that light passes through the object.

B. **Use of the Low Power.**—With the low power objective in line with the tube, and the eye on a level with the stage, turn the coarse adjusting screw so as to lower the tube until the objective is nearer the object than the stated focal distance (usually $\frac{2}{3}$ inch). With one eye at the ocular looking down through the tube, turn the coarse adjusting screw slowly so as to *raise* the objective until the object comes clearly into view. With the coarse adjustment focus carefully.

Move the slide about so as to see all parts of the object. Observe the apparent reversal of direction of the motion, and the inversion of the image. Are right and left also reversed? Open and close the diaphragm slowly, observing the effect. The best results are secured with an aperture barely large enough to admit sufficient light.

In working with the low power it is usually best to use only the coarse adjustment for focusing; but when only slight changes of focus are needed these may be secured with the fine adjustment. Test the action of the fine adjustment and observe that it works slowly and within a very limited range; the screw must not be turned more than one or two complete rotations in the same direction.

C. **Use of the High Power.**—Before using the high power always see that the object is in perfect focus and *in the exact center of the field of view* with the low power. Then turn the high power objective into place. If the microscope is in perfect adjustment (the two lenses parfocal), the object will now be in focus with the high power. But if, as is often the case, the lenses are not strictly parfocal, place the eye at the level of the stage and very carefully lower the tube by means of the coarse adjustment until the objective almost touches the cover glass of the object slide—that is, nearer than the focal distance of the high power objective (usually $\frac{1}{6}$ inch). With the eye at the ocular then raise the tube slowly until the object comes clearly into view; finish the adjustment with the fine screw. Readjust mirror and diaphragm until best results are obtained. While

working with the high power keep the fingers on the fine adjusting screw and turn it very slightly, alternating the direction so as to focus on all depths of the object.

D. The following points should be especially noted:

1. The object to be examined should usually be mounted in a liquid and covered with a cover glass—especially if it is to be studied with the high power.

2. Always study the object first with the low power in order to obtain a general view. Never use the high power when the low power will do—there are many disadvantages attending the use of the high power.

3. Never focus downward with the coarse adjustment while the eye is at the tube—the result usually is to smash the slide and perhaps also to injure the lens.

4. Do not allow the objective to come in contact with the cover glass, balsam or water used in mounting the object, or anything else except lens paper used in cleaning the lens. Be especially careful not to touch the lenses with the fingers. If the image is not clear this is usually due to dirty lenses. To clean a lens, breathe on it and wipe it dry with lens paper; if this is not sufficient speak to the instructor. (Balsam may be removed with xylol which should be wiped off with a clean soft cotton cloth.)

5. While working with the microscope, keep both eyes open; this will lessen eye strain.

6. While working with the high power the fine adjustment should be used almost constantly. This will save straining the eyes in an effort to see details out of focus, and will reveal the finer structures.

7. Careful adjustment of the diaphragm and manipulation of the mirror so as to throw some shadow into the field will often reveal structures that in too bright light are lost sight of. Oblique illumination is often useful.

8. Better results can be secured if the stage is kept level. The use of the clips for clamping the object slide in place is, as a rule, to be avoided, since it interferes with delicate manipulation of the slide.

9. In putting away the microscope see that the object slide is returned to the tray and not left on the stage or on your table. See that the low power objective is in position for use and that the clips are in place. Be sure to return the microscope to the place on the shelf indicated by its number.

THE FROG.

The following directions refer more particularly to Rana pipiens, but can be used with other species of frog if desired.

In the following pages, the terms **right** and **left** refer to the right and left sides of the frog, not of the observer. The term **dorsal** means the side that is ordinarily uppermost during life; **ventral** means the side that is ordinarily kept toward the earth. The **anterior** end of the body is the one pointing in the direction in which the animal ordinarily moves (*i. e.,* the head end, terminating in the snout), as opposed to the **posterior** end. **Medial** is used to indicate nearness to the middle line; **lateral** is the contrasting term. **Proximal** refers to that part of an organ or structure nearest to its center or to its attachment to the body; **distal** is the opposite adjective.

I. Attitudes and Movements.

Notice the normal resting position of the living frog, especially the attitudes of the fore and hind limbs. Induce a frog to leap; how is this movement effected? Are any preparatory movements required? What is the significance of the normal resting position?

Describe the attitude of a frog resting at the surface of deep water. How much of the frog is above water? What is the advantage of the protrusion or bulging of the eyes? Can the frog in this position secure air? When a frog in this position is alarmed, it will dive quickly to the bottom. Describe in detail the successive movements by which this is accomplished. What is the significance of the resting position at the surface of deep water?

Study the respiratory movements and explain fully how air is taken into and expelled from (*a*) the mouth, and (*b*) the lungs. The frog does not possess ribs and is unable to enlarge the cavity of the body directly; hence it employs an indirect method of filling the lungs, which can be made out by a careful study of the move-

ments of (*a*) the floor of the mouth, (*b*) the nares (nasal openings), and (*c*) the body. The glottis, through which air passes from the throat into the lungs, may be opened or closed as occasion demands.

Rotate a frog in a horizontal plane. Observe that the head turns in a direction opposite to that of the movement of the plate on which the animal is resting; often the body also turns about in the same way. Rotate the frog back and forth about a horizontal axis and observe that the head is moved up and down contrary to the direction of rotation. These movements are called **compensatory movements,** and they serve to maintain a fixed orientation in space.

II. External Characters.

The following study should be made chiefly on the living frog. Preserved specimens in addition are desirable.

Notice the division of the entire animal into head, trunk and limbs. Is there a distinct neck? Is the frog characterized by bilateral symmetry? antero-posterior differentiation? dorso-ventral differentiation? Observe the "hump" on the frog's back; examine a skeleton and discover how the hump is produced.

Observe carefully the color and color pattern of the dorsal and ventral sides respectively. Is the coloration such that it would be likely to afford protection to the animal in its natural environment? Observe that the skin is smooth, moist and slimy; of what advantage is this to the animal? Besides mucus, the skin of the frog, when irritated, secretes a small amount of a whitish, slightly poisonous fluid; in the toad the glands secreting this fluid are very well developed. In the frog observe:

1. The **eyes;** observe that they are ordinarily protruded, but are retracted when the lids are closed. There are short **upper** and **lower eyelids,** and in connection with the lower eyelid a broad semi-transparent **nictitating membrane,** which functions as a supplementary lower eyelid. (The nictitating membrane reaches its highest development in certain reptiles and in birds, while in mammals, including man, it is represented by a rudimentary fold at the inner angle of the eye.) The outer or exposed portion of the eyeball is covered with a transparent mem-

brane known as the **cornea.** The **iris** is a pigmented ring situated behind the cornea and showing through; it acts as a diaphragm, limiting the amount of light that enters the eye. The **pupil,** through which light enters the eye, is an oval aperture surrounded by the iris. Watch the eye carefully to see if the pupil changes in size through the expansion and contraction of the iris.

2. The **tympanic membrane** of the ear, a short distance behind the eye.

3. The **brow spot,** a very small light spot midway between the anterior ends of the eyes. The brow spot is the vestige of a median eye which was functional in ancestral forms.

4. The **mouth.** This is kept tightly closed, except in the act of taking food.

5. The **anus** (posterior opening of the digestive tube). It is slightly dorsal in position.

6. The **anterior nares,** or external openings of the nasal cavities.

7. The **fore limbs.** There are three divisions, **upper-arm, fore-arm** and **hand (manus).** The **wrist,** forming the proximal portion of the manus, is scarcely distinguishable externally. In the male there is a thickening along the inner edge of the first finger or **digit,** which is especially prominent during the breeding season.

8. The **hind limbs.** There are three divisions, **thigh, leg** and **foot (pes).** Certain bones of the proximal part of the foot (the **ankle**) are greatly elongated; of what advantage is this to the frog? What is the function of the web of the hind foot?

III. The Mouth Cavity.

For the following study both recently killed and preserved specimens are desirable.

Open the mouth of a frog to its fullest extent and observe:

1. On the roof:

(*a*) The **teeth.**

(1) **Maxillary teeth** on the edge of the upper jaw.

(2) **Vomerine teeth,** two groups on the fore part of the roof. Count them with the aid of a dissecting needle; about how many teeth in each group? Is the number constant in different frogs?

(3) Using a dry prepared skull, examine a maxillary tooth carefully with a hand lens. Distinguish **crown** and **root.** How are the teeth inserted on the jaw bone (*i. e.,* in sockets or otherwise)? Do they offer more resistance to an object entering or leaving the mouth (test with the finger)? Are they adapted for biting, for chewing, or merely for holding the prey?

(*b*) The **posterior nares** (internal openings of the nasal or olfactory sacs), lying lateral to the vomerine teeth. Pass a bristle through the anterior nares and notice where it emerges into the mouth.

(*c*) The **Eustachian tubes,** a pair of cavities at the sides of the posterior part of the roof; where do they lead?

(*d*) A pair of rounded prominences in front of the Eustachian tubes; how are they caused? Press one of the eyes with your finger, and observe that the eye may be made to protrude into the mouth cavity.

2. On the floor:

(*a*) The **lower jaws** forming the margin. Do they bear teeth?

(*b*) The **floor** proper is soft and fleshy, and slightly stiffened by the cartilaginous body of the hyoid apparatus (examine a preparation of the hyoid apparatus).

(*c*) The **tongue.** Observe its shape and manner of attachment. In a fresh specimen observe that the tongue is sticky; of what advantage is this to the animal? Examine a preparation of a frog with the tongue protruded. If possible observe the action of the tongue in a hungry frog which may be induced to snap at a small piece of red cloth dangled before it with a cord. The tongue is of use in capturing small animals (*e. g.,* insects).

(*d*) The **glottis,** a longitudinal slit on the summit of a prominence far back in the throat region. Force it open with a seeker; the cavity into which it leads communicates with the lungs.

(*e*) The opening of the **œsophagus,** a distensible tube leading from the posterior part of the mouth cavity to the stomach.

(*f*) In the male: the openings of the **vocal sacs** just within the angle of the mouth.

The posterior part of the mouth cavity, into which the glottis and the Eustachian tubes open, is called the **pharynx.**

Sketch the floor and the roof of the mouth, twice natural size, labelling all the parts.

IV. Preliminary Study of the Internal Organs.

The work of this section should be done on a freshly-killed specimen. The method of cutting the frog open will be demonstrated at each table, but each student should perform the operation on his own specimen. The inflation of the lungs and the bladder will be demonstrated.

Place the frog on its back and with the smaller pair of scissors divide the skin in the ventral median line from the posterior to the anterior end of the body.

How and where on the ventral surface is the skin attached? Observe the great **lymph spaces** beneath the skin, between the attachments. Turn the flaps of skin outward and pin them to the tray; to do this it will be necessary to sever certain of the attachments.

Note (by feeling) the cross-shaped **pectoral girdle,** the center of which lies in the line uniting the bases of the two arms (examine the pectoral girdle in a preparation of the skeleton of the frog). The longitudinal bar of the cross is the **sternum.**

Make a longitudinal incision through the ventral body-wall, a little to the left of the median line and extending from the posterior end of the body to the hinder end of the sternum; be careful not to cut the vein running along the median line. Be careful also not to injure the bladder (described below). Observe the **anterior abdominal vein** in the middle line on the under surface of the larger flap.

With your stronger pair of scissors carry the incision forward through the pectoral girdle to the level of a line joining the angles of the jaws.

Under the sternum, which can be raised, is a membranous sac, the **pericardium,** containing the heart. Observe the position of the pericardium with respect to the large dark-colored lobed organ, the **liver,** filling the front part of the body cavity. Observe the attachment of the pericardium to the ventral body-wall by a vertical membrane which also supports the liver. What relation does this membrane bear to the anterior abdominal vein?

Trace this vein backward and forward until it leaves the body wall, and find where it terminates anteriorly.

After carefully freeing the sternum from the pericardium, pin back the flaps of the body wall so as to expose the internal organs (viscera). Find the following organs:

1. The **heart,** already noticed. In a freshly killed frog the heart usually continues to pulsate for a considerable time. The large **veins** entering the heart will be found dark-colored because filled with blood; the **arteries** are white because after death they contain little blood.

2. The **digestive system.** (*a*) The **alimentary canal** or digestive tube is composed of the following parts:

(1) The **œsophagus,** a short, wide tube, very distensible, leads from the mouth cavity to the stomach. Find its opening into the mouth cavity, and insert a probe through the mouth cavity into the stomach.

(2) The **stomach,** a whitish organ, wider and with thicker walls than the rest of the alimentary canal. Its anterior end is known as the **cardiac** end and its posterior end as the **pyloric** end. Observe the pyloric constriction; what is its function? Cut open the stomach by a longitudinal incision on its *left* side, wash out the contents and observe the folds of the inner layer or **mucosa.** Examine the cut edge with a lens and note just outside of the mucosa a spongy layer, the **sub-mucosa,** which is surrounded by a third layer, the **muscular layer,** and this in turn is covered externally by the very thin glistening **peritoneum.**

(3) The **small intestine,** into which the stomach is continued at its pyloric end. The first portion, which runs anteriorly, is the **duodenum.** Under cover of the liver the duodenum bends posteriorly and the part of the small intestine posterior to the bend is known as the **ileum.** The latter, after a number of coils, passes abruptly into:

(4) The **large intestine.** The posterior part of the large intestine is called the **cloaca;** this opens to the exterior by the **anus.**

If the animal has not been too long dead, observe the **peristaltic movements** of the intestine (pinching the small intestine with fine forceps will usually start these movements). What is the function of these peristaltic movements? The entire alimentary canal

is fastened by a thin membrane, the **mesentery,** to the dorsal body-wall.

(*b*) The **liver,** already noticed, is a large dark-red organ partly covering the duodenum and stomach. How many lobes has it? On the dorsal side observe the **gall bladder** or **bile sac** which is usually filled with green **bile.** Observe the **bile duct** which extends from the gall bladder to the duodenum, and the various **hepatic ducts** which enter the bile duct from the liver. In a freshly-killed specimen, by gently pressing the gall bladder and forcing bile from it into the duct, the course of the bile duct may be more easily followed.

(*c*) The **pancreas,** a flat, irregularly lobed yellowish organ lying between the stomach and the duodenum and stretching from the liver to the small intestine. The bile duct passes through it, and the pancreatic ducts empty into the bile duct. Look up the course of the bile duct in the human body; does it pass through the pancreas as in the frog?

3. The **lungs,** two thin-walled sacs lateral and posterior to the heart; they lie dorsal to the liver and are usually hidden by it. Insert a blow-pipe into the glottis of a freshly-killed frog and inflate the lungs.

4. The **spleen,** a round dark-red organ in the mesentery near the anterior end of the large intestine.

5. The **excretory system.** (*a*) The **kidneys,** a pair of reddish, flat bodies lying next to the dorsal wall of the body, and separated from the body cavity by a thin membrane, the **peritoneum.** Expose a kidney by cutting through the peritoneum of one side only. If the specimen is a female, in order to expose the kidney it may be necessary to remove one ovary (described below) by cutting the membrane by which it is attached. Observe a yellowish band, the **adrenal body,** on the ventral surface of each kidney; this corresponds to the **suprarenal body** of man.

(*b*) The **ureters** emerge from the posterior fourth of each kidney on its lateral edge. They are slender, white tubes, one for each kidney, which converge as they pass posteriorly, and empty into the dorsal side of the cloaca.

(*c*) The **bladder,** a slightly bi-lobed sac opening by a narrow neck into the ventral side of the cloaca. Insert a blow-pipe or

a large pipette through the anus of a freshly-killed frog and inflate the bladder.

6. The **corpora adiposa,** or **fat bodies,** yellow tufts of flattened finger-shaped processes attached to the dorsal body-wall near the kidneys and just anterior to the gonads (see below). They vary greatly in size in different specimens.

7. The **reproductive system.** This consists of the **gonads** which produce the sex cells, and the ducts by which the sex cells are carried to the outside. In order to make a study of both male and female reproductive systems, supplement the study of your own frog by the examination of demonstration specimens.

In the male observe the **testis** (male gonad), an oval light yellow body near the ventral surface of the kidney; at its anterior end it is closely attached to the fat body. In the membrane which supports the testis note the **vasa efferentia,** a number of delicate tubes leading from the testis to the inner margin of the kidney; here they enter a longitudinal canal in the kidney, and from this canal the spermatozoa (male sex cells) reach the ureter by way of the urinary tubules in the kidney. Thus in the male frog, the ureters function also as **vasa deferentia.** Observe the slight dilation of the ureter which functions as a **seminal vesicle.** In the male of one species of frog (Rana pipiens) the **Muellerian duct,** the homologue of the oviduct of the female, persists as a conspicuous tube extending forward from the cloaca. In the males of most forms this is represented by a mere rudiment or is absent entirely.

In the female observe: (*a*) The **ovaries,** usually large organs, filled with eggs and especially well developed during the breeding season. Observe their relation to the fat bodies and the manner in which the ovaries are suspended in the body cavity. Notice the thin membrane (peritoneum) that covers the ovary and is continued into its dorsal support. After the discharge of the eggs the ovary becomes much reduced in size.

(*b*) The **oviducts,** a pair of much convoluted tubes lying in the sides of the body cavity. In the breeding season they become thick and glandular, furnishing the jelly which surrounds the eggs. At its anterior end each oviduct opens, by a wide funnel-shaped mouth, into the body cavity near the base of the lung.

Pass a bristle into the anterior end of the oviduct. Posteriorly each oviduct passes into a thin-walled distensible portion, the **uterus;** this opens into the cloaca. How are the oviducts attached to the body wall?

V. The Peritoneum.

The space between the alimentary canal and the body wall is known as the **cœlome,** or **body-cavity,** and the thin membrane which lines the body cavity is the **peritoneum.** Near the mid-dorsal line the peritoneum is deeply infolded to surround the intestine, which lies within the edge of the fold. Between the intestine and the dorsal body wall the two layers of the fold are in contact, forming the **mesentery** which serves to support the intestine and bind the several coils together. The abdominal viscera (intestine, etc.) are really outside of the peritoneum which forms a closed sac into which the viscera appear as if pushed from the outside. Observe the peritoneal covering of the pericardium, liver, gall-bladder, and the vertical mesentery (severed during the preliminary dissection) which unites the liver to the ventral body wall. The ovaries, testes and oviducts possess special mesenteries; examine them. The bladder is attached by a short mesentery to the ventral side of the rectum; notice the other mesenterial attachment of the bladder. The lungs project from in front into the body cavity and therefore possess a complete investment of peritoneum. The blood vessels of the viscera run within the mesenteries. The kidneys lie dorsal to the peritoneum, within the great lymph space between the peritoneum and the dorsal body wall.

Study the course of the peritoneum in the diagram of a cross-section of the frog's body shown on page 78 of Holmes' Biology of the Frog. Make a similar *diagram* of a cross-section of the body of a *female* frog. (This diagram should be about 10 cm. in diameter.) If your specimen is a male, consult the model showing the internal organs of a female frog, also a preparation of the female reproductive system.

VI. The Digestive System.

A. **General Anatomy.**—Review your observations on the anatomy of the digestive system and make an outline *drawing* ($\times$ 2)

of the alimentary canal (œsophagus, stomach, small and large intestine), showing in addition the liver, gall bladder (bile sac), pancreas and the course of the bile duct. The liver should be turned forward to expose the pancreas, etc. Examine a preparation of the digestive system with the organs arranged in the proper position for sketching.

B. **The Finer Structure of the Alimentary Canal.**

1. **Cross-section of the small intestine.** (*a*) Under low power observe the shape of the cavity, and the five main layers of the wall, beginning from within:

(1) The **mucosa,** or **alimentary epithelium.** Is there more than one layer of cells? Notice the closely packed nuclei. Notice the **goblet cells** scattered among the others; they may be recognized by the clear oval mass in their outer ends.

(2) The **submucosa,** a layer consisting mainly of connective tissue (characterized by occasional small rounded cell-bodies and a large amount of loose fibrous intercellular substance) follows the folds of the mucosa. Within the submucosa are occasionally found large irregular **lymph spaces.**

(3) The **circular layer of non-striated muscle.** This consists of slender spindle-shaped fibers each with an elongated nucleus.

(4) The **longitudinal layer of non-striated muscle.** The fibers have the same structure as those of the circular layer, but are cut transversely.

(5) The **peritoneum** or **serous layer** consists of a single layer of flattened cells cut transversely so that each cell appears spindle-shaped in the section. This layer forms the outer covering of the alimentary canal.

(*b*) To show the finer structure *draw* only a small part of the section under high power. Select for this purpose a portion of the wall of the intestine including a small fold, and showing the above characteristic features as clearly as possible.

2. **Cross-section of the stomach.** Study a transverse section taken through the cardiac region of the stomach. Under low power, identify and study the following layers, which are named in their order from within outward.

(*a*) The **mucosa.** This is the innermost layer of the stomach and is so folded as to form long tubular glands set very closely

together. Study carefully one of these glands; note three regions distinguished by the different character of the cells. The three regions may be called **mouth, neck** and **body** respectively. Under high power, *draw* a gland showing clearly the character of the cells in the different parts; the drawing should be about 8 or 10 centimeters in length.

(*b*) The **submucosa.** Examine under high power. The submucosa is much thicker than in the small intestine. Along its inner margin, adjacent to the mucosa, observe a narrow zone called the **muscularis mucosæ,** consisting of an inner circular and an outer longitudinal layer of non-striated muscle fibers. (In the intestine a muscularis mucosa is sometimes found, but never well developed.)

(*c*) The **muscular layers.** Compare with those of the small intestine.

(*d*) The **peritoneum,** as in sections of the intestine.

VII. The Urogenital System.

A. **General Anatomy.**—Review your observations on the excretory and reproductive systems (**urogenital system**) of both sexes, supplementing the study of your own specimen by the examination of permanent preparations. Make an outline *sketch* of the urogenital system of your own frog, including in your drawing the large intestine and the cloaca in order to show the connection of the ducts and the bladder with the cloaca. If the specimen is a female it will be necessary to remove the ovary of one side, if this has not already been done, in order to expose the kidneys and ureters; this ovary may be omitted from the drawing. On account of their intimate relation to the reproductive system, the fat bodies may be included in the drawing. This drawing should be about twice natural size.

How do the eggs produced in the ovary of the female reach the exterior?

B. **The Finer Structure of the Kidneys.**—In transverse sections of a kidney distinguish median and lateral edges, dorsal and ventral surfaces. The dorsal surface is usually more convex, the lateral edge more acute.

In a cross-section, find the **ureter;** this lies on the dorsal side

close to the lateral edge, and may be distinguished from neighboring blood-vessels by the columnar character of the epithelium lining its walls, and by the absence of blood-corpuscles.

The **Malpighian corpuscles** lie nearer to the ventral than to the dorsal surface; each consists of a rounded or oval body, the **glomerulus,** formed by a cluster of capillaries and nearly surrounded by an open space limited by a thin membrane (Bowman's capsule). At one point the glomerulus is suspended by a short slender stalk which naturally does not appear in all the sections.

The **uriniferous tubules** are cut in various planes; observe the cubical or columnar epithelium lining them. Each tubule starts in a capsule (usually opposite the stalk of the glomerulus) and after a complicated course opens into the ureter. Try to find the beginning of a tubule, where it connects with the capsule.

Under high power, *draw* a portion of a cross-section of the kidney showing a Malpighian corpuscle and some adjacent tubules; if possible, select for this purpose a section through a Malpighian corpuscle showing one or both of the following features: (*a*) the stalk by which the glomerulus is suspended within the capsule, and (*b*) the connection of a tubule with the capsule.

Look for **nephrostomes** (funnel-like openings lined with cilia) on the ventral surface of the kidney. *Draw* a nephrostome under high power.

VIII. The Circulatory System.

A. **The Blood.**—Place a drop of blood on a slide; smear the edge of a cover slip with vaseline and place it over the drop; the vaseline will prevent evaporation. Under the low power observe that the blood consists of a fluid portion, the **plasma,** and solid elements, the **corpuscles.** Under the high power observe that the corpuscles are of two kinds, as follows:

1. **Red corpuscles,** elliptical discs tinged slightly red with **hæmoglobin.** Are they thicker at the margin or in the center? Note the central nucleus. *Draw* a corpuscle as seen from the broad side; if possible find and *draw* an edge view. Each drawing should be at least two centimeters in its longer diameter.

2. **White corpuscles (leucocytes),** generally about one-third the size of the red; they are best seen by making the opening of

the diaphragm of the microscope very small. The white corpuscles vary considerably in size, form and structure. The outline is sometimes irregular and undergoes amœboid changes. The usually large granular nucleus may be either spherical or irregular in form; the cytoplasm may be either clear or granular. Often the cytoplasm is very granular so that the nucleus cannot be seen. *Draw* several white corpuscles differing in form or structure. If one is found actively changing in form, sketch it at intervals of about one-half minute, to show changes in form.

B. **The Heart.**—The heart has the form of a cone with its apex directed posteriorly. The posterior part (**ventricle**) is thick-walled and opaque; the anterior part (**auricles**) is thin-walled and usually filled with clotted blood. Observe the large vessel, the **truncus arteriosus,** arising from the right anterior corner of the triangular ventricle; it passes anteriorly and to the left over the ventral surface of the auricles and branches immediately into the two **aortic arches.** *Sketch* a ventral view showing ventricle, auricles, truncus arteriosus and aortic arches; label all parts. Make this drawing small ($\times 2$) and place it in the center of a clean page so that the arterial system may be added later.

Gently lifting the apex of the heart, examine the dorsal surface carefully from behind and from the sides; notice the thin-walled **sinus venosus,** one of the divisions of the heart, filled with clotted blood, dorsal to the auricles. Three veins enter it: the **posterior vena cava** from behind, the **right** and **left anterior venæ cavæ** separately from in front. These veins, especially the posterior vena cava, are large, though like all the veins they have thin walls. The small **pulmonary veins** may also be seen arising from the inner faces of the lungs and uniting to form a single trunk, the short **pulmonary vein** proper, which enters the left auricle.

With scissors cut out the heart, being very careful to leave the stumps of all the blood vessels attached, and place in a small dish of water. Carefully clean bits of membrane, etc., from the surface of the sinus venosus. Notice the triangular form of the sinus and identify the three veins (venæ cavæ) entering the three angles. If you are unable to recognize these parts, consult figure 73 of Holmes' Biology of the Frog. The pulmonary vein enters

the left auricle just in front of the anterior boundary of the sinus. Inserting the point of your scissors into the posterior vena cava make two diverging cuts running to the base of each anterior vena cava. Turn back the flap thus formed, wash out the clotted blood and examine the interior of the sinus. The large transverse opening near the anterior end leads into the right auricle.

Examine a preparation of a heart dissected from the ventral surface; compare figure 72 of Holmes' Biology of the Frog. Make a similar preparation for yourself by cutting away the ventral wall with scissors or a very sharp scalpel and exposing the following parts, all of which should be identified:

1. The two **auricles,** right and left. Find the **interauricular septum** or thin membrane separating the two. Which auricle is the larger? Find the opening of the sinus venosus into the right auricle, and of the pulmonary vein into the left; notice the valves at the opening of the sinus. How do the valves function?

2. On each side of the interauricular septum observe the openings of the auricle into the ventricle; these openings are guarded by valves.

3. The cavity of the ventricle. Observe the spongy character of the walls, the spaces of which are continuous with the central cavity of the ventricle.

4. The opening into the truncus arteriosus guarded by three pocket-like **semi-lunar valves.**

5. The **spiral valve** attached to the wall of the truncus arteriosus. How does it end anteriorly and posteriorly?

6. The **aortic arches.** Cut one across not far from its origin. Observe that the cavity is divided by two longitudinal partitions into three parallel tubes. Observe how these three tubes enter the truncus; notice especially the entrance of the pulmo-cutaneous arch (see figure 73 of Holmes' Biology of the Frog).

If possible, study the beating of the heart in a freshly killed frog. In what order do the various parts contract?

C. **The Arteries.**—These convey blood away from the heart. A *diagram* ($\times$ 2) of the arterial system should be made showing all the arteries mentioned below. First examine a preparation or a model showing the principal arteries; then study an injected

specimen. With fine scissors cut away the pericardium from the aortic arches, then with fine forceps carefully pick away the muscles and other tissues wherever necessary to expose the arteries. If the specimen is a female it may be necessary to remove the ovary and oviduct from one side. If possible leave the specimen in such condition that it may be used for the study of the arteries by future classes.

The three subdivisions of the aortic arches are continued on each side into the main arterial trunks, the **carotid arch,** the **systemic arch,** and the **pulmo-cutaneous arch.**

1. The **carotid arch** is the most anterior of the three and is formed by the **common carotid** artery. A short distance from its origin it divides into the small **external carotid** or **lingual** artery and the **internal carotid** artery.

The **lingual artery** passes forward, giving off branches to the thyroid gland, various muscles of the hyoid apparatus, and the tongue. Trace it after removing the skin and superficial muscles from the ventral surface of the floor of the mouth. The lingual artery often fails to be injected satisfactorily.

The **internal carotid** artery. Just beyond the lingual artery the internal carotid enters a small swelling of spongy structure known as the **carotid gland.** This organ, which often blocks the injection, is pigmented; beyond it the internal carotid turns first dorsalward, then anteriorly above the skin of the roof of the mouth to the region of the eye. To trace its distribution in this region pry open the mouth of the frog and make a longitudinal slit in the median line through the skin of the roof of the mouth. Turn back the flaps and observe the internal carotid artery coming forward to the region of the eye. Here it breaks up into a number of smaller arteries, the most important of which are the **palatine,** which courses forward along the roof of the mouth; the **ophthalmic,** which passes directly to the eye (find it by dissecting away the large eye muscle already exposed); and the **cerebral carotid,** which cannot readily be traced since it passes directly into the cranium and supplies the brain.

2. The **systemic arch** is the middle one of the three main arches. It passes dorsalward above the œsophagus and posteriorly, giving off branches on its way, to meet its fellow with which

it unites in the median line to form the **dorsal aorta.** The dorsal aorta passes posteriorly, giving off branches to the viscera and body wall, and bifurcates near the posterior end of the body cavity.

(*a*) **Branches of the systemic arch.**

The small **laryngeal** artery arises from the dorsal surface of the systemic arch near its origin. It is distributed to the larynx and pharynx.

The **occipito-vertebral** artery arises from the systemic arch at about the level of the third vertebra; it passes anteriorly and turns dorsalward at the level of the posterior end of the cranium, sending one branch anteriorly to the cranium and another posteriorly along the vertebral column. To trace the course of these branches turn the frog dorsal surface up, slit up the skin a little to one side of the dorsal median line from the tip of the nose to the "hump" of the frog's back, and reflect the more lateral flap. Dissect away the suprascapula (expanded dorsal portion of the shoulder skeleton) and the muscles covering the dorsal surface of the vertebræ. The occipito-vertebral artery will be seen emerging from the body cavity just posterior to the skull; it divides into an anterior branch, the **occipital** artery, and a posterior branch, the **vertebral** artery. Trace the course of each.

The small **œsophageal** artery arises near the angle between the systemic arch and the occipito-vertebral or sometimes from the latter. It passes to the dorsal surface of the œsophagus.

The **subclavian** artery arises just posterior to the occipito-vertebral and passes laterally to supply the muscles of the shoulder and arm. On its course toward the arm it sends off several small branches to neighboring muscles and the body wall.

(*b*) **Branches of the dorsal aorta.**

The **cœliaco-mesenteric** artery is a large artery arising near the junction of the systemic arches; it divides into the **cœliac** and the **anterior mesenteric** arteries. The cœliac artery gives off the **left gastric** artery and finally divides into the hepatic and **right gastric** arteries; trace their distribution. The anterior mesenteric artery sends branches to the spleen (**splenic** artery) and small and large intestines.

The **urogenital** arteries are four or five (or even more) small arteries which arise from the ventral surface of the dorsal aorta

and supply the kidneys, fat-bodies, sexual organs and the ducts of the urogenital system. Those arteries that are distributed to the kidneys alone are sometimes called **renal** arteries.

The **lumbar** arteries are small vessels, variable in number and position, passing to the neighboring dorsal body wall and to the spinal cord.

The **posterior** or **inferior mesenteric** supplies the dorsal side of the rectum, and in the female sends branches to the uteri.

The large **iliac** arteries are produced by the bifurcation of the dorsal aorta. A short distance behind the bifurcation each artery gives off a branch which divides into an **epigastric** artery supplying the ventral body wall, and a **recto-vesical** to the rectum and bladder. Further on the iliac artery gives off the **femoral** artery to the lateral muscles of the body wall and the hip joints; it then continues down the thigh as the **sciatic** artery. The iliac artery and its branches can be studied from the body cavity. To expose the sciatic artery proceed as follows: Mark the point where the artery leaves the body cavity by thrusting a pin through the body wall to the dorsal surface. Remove the skin from the dorsal surface of the body and leg; dissect away the muscle and connective tissue posterior to the point indicated by the pin and expose the sciatic artery. Follow the sciatic artery down the dorsal side of the leg by forcing apart the great muscles in this region.

3. The **pulmo-cutaneous arch** is the most posterior of the aortic arches. A short distance from its origin it divides into two branches, as follows:

The **pulmonary** artery passes straight to the lung. At the root of the lung it divides into a number of branches which run toward the tip of the lung, each giving off branches to the interior trabeculæ.

The **cutaneous** artery at first turns laterally, slightly forward and dorsalward, crossing the systemic arch; it then turns sharply dorsalward. To trace its further course turn the frog on its ventral surface, turn back the flaps of skin on the dorsal surface, and carefully dissect away the muscle just posterior to the tympanum. The cutaneous artery will then be seen emerging from the body cavity just in front of the suprascapula. Here it divides

into three branches; trace their distribution. What evidence do you find that the skin of the frog is an important organ of respiration?

D. **The Capillaries, and their Relation to Arteries and Veins.**—Examine a microscopic demonstration of the circulation of the blood in the web of the frog's foot. Arteries may be distinguished from veins by the fact that the blood corpuscles scatter to enter the capillaries diverging from an artery, while in the veins the corpuscles accumulate from the capillaries converging to form the vein. A slight pulsation may sometimes be observed in the smaller arteries. In the capillaries the corpuscles usually advance in single file.

E. **The Veins.**—For the following study use an entire uninjected specimen; if possible leave the specimen in such condition that it may be used for the study of the veins by future classes. The veins may be distinguished from the arteries by the fact that the former are usually filled with dark-colored blood. As an aid to the identification of the veins the student may consult figure 75 of Holmes' Biology of the Frog. A drawing is not required.

The veins convey blood from the capillaries toward the heart. We have seen that the blood reaches the heart through four vessels, viz., two anterior venæ cavæ, the posterior vena cava, and the pulmonary vein. The anterior venæ cavæ receive blood from the head, arms, and anterior part of the body generally; the posterior vena cava receives blood from the viscera (excluding the lungs) and the posterior part of the body; the pulmonary vein, through its two branches, receives blood from the lungs.

1. **Vessels which enter the anterior venæ cavæ.**

(*a*) The **external jugular** entering anteriorly is formed by the union of the **lingual** vein, conveying blood from the floor of the mouth and the tongue, and the **mandibular** vein, running ventral to the lower jaw.

(*b*) The **innominate** vein entering laterally is formed by the union of the **internal jugular,** conveying blood from the interior of the skull, and the **subscapular,** conveying blood from the dorsal side of the arm and the shoulder.

(*c*) The **subclavian** vein, entering laterally and posteriorly, is formed by the union of the **brachial** vein, returning blood from

the arm, and the **musculo-cutaneous** vein, a large vein running in the skin along the side of the body and returning blood from the skin and muscles of the dorsal and lateral regions of the trunk and head.

2. **Vessels which enter the posterior vena cava.**

(*a*) The **hepatic** veins from the liver enter the posterior vena cava not far from the sinus venosus.

(*b*) The **renal** veins from the kidneys pass straight toward the middle line and form by their union the beginning of the posterior vena cava.

(*c*) The **genital** (**ovarian** or **spermatic**) veins from the ovaries or testes, according to the sex, lead into the posterior vena cava, either directly or by uniting with renal veins.

3. **The portal systems.** A portal system may be defined as a system of veins which carries blood from one network of capillaries to another in an entirely different organ. There are two portal systems in the frog: the **renal portal system** carrying blood from the hind legs to the kidneys, and the **hepatic portal system** conveying blood from several sources to the liver.

(*a*) The **renal portal system.** The **renal portal vein** enters the kidney from behind, coursing along its entire lateral edge. It receives the following branches:

The **dorso-lumbar** vein from the dorsal wall of the abdomen (in the female also from the oviduct) enters the renal portal vein near the middle of the kidney.

The **iliac** vein. This is the dorsal branch of the great **femoral** vein of the thigh and leg. The latter runs forward near the dorsal surface of the thigh past the hip-joint, then turns toward the ventral surface, dividing into two branches: the **pelvic** vein which unites with its fellow of the opposite side to form the **anterior abdominal** vein, and the iliac vein which passes dorsally and anteriorly to unite with

The **sciatic** vein. This, like the iliac vein, conveys blood from the thigh to the renal portal vein.

(*b*) The **hepatic portal system.** The liver receives venous blood from two sources: first, from the posterior limbs and bladder by means of the anterior abdominal vein; second, from the digestive system by means of the hepatic portal vein.

The **anterior abdominal** is formed by the union of the pelvic veins; immediately beyond this point it receives the **vesical** veins from the bladder. It passes thence forward along the mid ventral body wall, receiving branches from the body wall, and opposite the liver it ascends to enter the substance of the liver. Just before reaching the liver it divides into three branches, two for the right and left lobes respectively and a third communicating with the hepatic portal vein. As the anterior abdominal vein turns up toward the liver it receives a small branch, the **cardiac** vein, from the base of the truncus arteriosus.

The **hepatic portal** vein receives branches which bring blood from the stomach, intestine, spleen and pancreas.

F. After completing the above work on the circulatory system write in your notebook answers to the following questions:

Through what arteries must blood pass from the heart to reach the lungs, the skin of the sides of the body, the liver, the small intestine, the kidneys, the hind limbs?

Mention the veins (and other organs if any) through which the blood corpuscles must pass, before reaching the heart, from the following parts: the lungs, the skin of the sides of the body, the small intestine, the hind limbs (two routes).

How does the blood entering the sinus venosus from the anterior venæ cavæ compare with that entering from the posterior vena cava as regards: (1) oxygenation, (2) nutritive material, (3) waste products? State reasons for your answers.

Before leaving the study of the blood-vascular system be sure you have gained clear ideas concerning the following points: The mode of action of the heart, the function of all the valves, the mode by which oxygenated blood and the non-oxygenated blood are kept separate (consult Holmes' Biology of the Frog, pp. 277 to 279), the general course of the circulation, and the changes that occur in the blood in the different parts of the body. Observe the general conformity of the venous system to the arterial and account for the similarity.

G. If possible observe in a living frog the beating of the posterior pair of **lymph hearts** (see Holmes' Biology of the Frog, p. 281). These are situated just beneath the skin in a slight depression between each hip-joint and the dorsal median line. The

contraction of these hearts usually causes a slight pulsation of the skin, but it may be seen more distinctly in a freshly-killed specimen from which the skin of this region has been removed without injury to the hearts.

IX. The Respiratory System.

Respiration in the frog is not carried on exclusively by means of the lungs; the skin and the mouth cavity are important accessory organs of respiration.

The lungs communicate with the pharynx or posterior part of the mouth cavity by means of the glottis, which opens from the dorsal surface of a cartilaginous box, the **larynx,** whose cavity is continuous with the cavities of the lungs.

Returning to the study of your original specimen, remove the liver, taking care not to injure the adjacent parts. Remove the skin and muscle from the throat region and expose the **hyoid apparatus,** the body of which consists of a thin shield-shaped sheet of cartilage, lying just in front of the larynx. This cartilage serves for attachment of the tongue and several muscles that move the floor of the mouth; a pair of strong bony processes, the **thyrohyoid processes,** extend posteriorly and support the larynx which lies between them. After examining a preparation of the hyoid, larynx and lungs, remove these organs as follows: cut the œsophagus across about 5 mm. behind the base of the lungs and, inserting one blade of the scissors in the open end, cut forward along the side toward the angle of the jaw and dorsal to the root of the lung of that side, taking care not to injure the lung. Continue this cut forward through the floor of the mouth close to the jaw to a short distance in front of the hyoid apparatus; make a transverse cut here and a cut similar to the first down the other side of the œsophagus. Remove the piece cut out and place it, ventral surface up, in a dish of water. Taking care not to injure the **thyroid glands** (see Section X), clear away the muscles in case this has not already been done completely, and observe the **larynx** embraced by the thyrohyoid process of the hyoid. The skeleton of the larynx is composed of the **cricoid** and the **arytenoid** cartilages. The cricoid cartilage consists of a slender ring surrounding the larynx and lying in nearly the same

plane as the thyrohyoid process, to which it is closely attached; at its posterior end it is produced into a median process or spine. From near its middle the cricoid cartilage gives rise to a transverse ventral loop, the **tracheal process,** which serves as a means of attachment for the lungs. The arytenoid cartilages are a pair of semi-lunar valves, whose apposed edges form the lips of the glottis; they afford attachment to muscles by which the glottis may be opened and closed.

Cut through the transparent membranous ventral wall of the larynx and expose the interior. Observe the **vocal cords,** two strong membranes attached to the anterior and posterior walls of the larynx; the inner edges are free and may be set in vibration by the expulsion of air from the lungs, thus producing the frog's peculiar croaking sound. The vocal apparatus of the male is larger than that of the female.

Cut open one of the lungs by a longitudinal incision, pin it out under water in a small dissecting tray, examine it with a lens and *sketch* a small portion of its inner surface. For this purpose a lung that has been preserved while slightly inflated is desirable.

Of what importance in the respiration of the frog is the hyoid apparatus?

X. The Thyroid Glands.

On each side of the hyoid apparatus, between the lateral process and the proximal end of the thyrohyoid process, is a small body known as the **thyroid gland.** For the exact location of these glands see figure 60 of Holmes' Biology of the Frog. Examine a gland under the low power of the microscope, observing the vesicles of which it is mainly composed. Study a prepared section of the thyroid. Observe the layer of epithelial cells lining each vesicle; the space between the vesicles is filled with connective tissue and blood vessels. *Sketch* a small portion of the section under high power.

XI. The Integument.

A. Mount on a slide a small piece of the outer or horny layer (**stratum corneum**) that has been shed from the skin. Such pieces may usually be found in the water in which living or

preserved specimens are kept. Be sure that you have obtained a portion only one cell in thickness; this must be mounted in water, and covered with a cover slip. The cells of this layer are much flattened and, as a rule, polygonal in outline; each of the polygonal cells contains a nucleus near its center. Among the ordinary epithelial cells of the stratum corneum may be found occasional smaller cells with rounded outline and a central tri-radiate opening; these are the **stoma cells,** through which the mucus secreted by the glands of the skin escapes to the exterior. *Sketch* a few cells of the stratum corneum, including a stoma cell.

B. With a hand lens study the skin of the frog, observing the differently colored pigments and the small papillæ, formed by groups of poison glands and mucus glands, found most abundantly on the dorsal surface. In the lighter areas the black pigment may be seen to be grouped in small irregular spots formed by the pigment cells.

C. Cut out a piece of the integument containing a moderate amount of pigment, scrape its inner surface in order to render it thinner, mount it on a slide with a little water and cover it with a cover slip. Observe, on focusing upon the outer surface, the cells of the horny layer with here and there small round transparent areas marking the location of the minute stoma cells with their tri-radiate openings. Underneath the stoma cells lie **mucus glands** whose ducts lead to the surface at the tri-radiate openings. In one of the deeper layers of the skin, the **cutis,** numerous **black pigment cells** will be found; some of these may be much extended and their processes profusely branched, while others appear to be in a much contracted condition. These differences are best seen by comparing the skin of a dark frog with that of a light one. The changes in these pigment cells cause the color changes in the skin. *Sketch* a black pigment cell showing numerous branches, and another in the contracted condition. Study the skin both by reflected and by transmitted light; do you find any other kind of pigment cells besides black ones?

D. Study a **cross-section of the skin.** The skin is divided into two principal layers, the outer or **epidermis** and the inner or **cutis.**

1. In the **epidermis,** which consists of several layers of cells,

observe that the outermost layer (stratum corneum) is composed of extremely flattened cells. This is the horny layer, described above, which is periodically shed. The inner or **Malpighian layer** is composed of several layers of cells; the innermost of these consists of columnar cells; the other layers show transitional stages between the latter and the stratum corneum.

2. The **cutis** is composed mainly of connective tissue in which are embedded numerous glands; it is roughly divisible into the following three layers:

(*a*) The **outer layer,** composed mainly of loose connective tissue, contains considerable pigment especially next to the Malpighian layer. Between and above the branched cells containing black pigment observe the **golden pigment cells,** which are oval granular cells containing, in life, the yellow pigment which gives the golden color to the skin.

The glands are mainly confined to this layer. Each gland is flask-shaped and its body is lined by a layer of thick secreting epithelial cells. If the section is cut near the middle of the gland the neck may be seen extending through the epidermis to the surface; the cells of the neck are flattened but they form a layer continuous with the secreting cells of the gland. The glands are of two kinds, the numerous small **mucus glands,** and the large **poison glands,** much fewer in number. The poison glands are distinguished by their granular contents.

(*b*) The **middle layer** is composed mainly of a compact mass of connective tissue fibers which run in a wavy course parallel to the surface of the skin. At intervals, transverse bands of connective tissue fibers cross this layer and extend into the outer layer. Both outer and middle layers are occasionally traversed by small bundles of non-striated muscle fibers extending between the glands.

(*c*) The **inner layer** is comparatively thin and composed of a loose meshwork of connective tissue and blood vessels. This layer is connected only at wide intervals with a similar layer of connective tissue which covers the skeletal muscles, the two layers being separated by great lymph spaces except in the septa where they become continuous.

Sketch a cross-section of the skin, selecting for this purpose a

portion that shows clearly as many of the above features as possible. Be sure to include a mucus gland, if possible one showing the neck extending through the epidermis.

XII. Epithelium.

An example of **flattened** or **squamous epithelium** is furnished by the stratum corneum of the integument. Simple **columnar epithelium** is found in the mucosa of the intestine. **Ciliated epithelium** is found lining the œsophagus and the roof of the mouth of the frog, and may be demonstrated as follows:

Gently scrape the roof of the mouth of a recently-killed frog, transfer the scrapings to a slide and mount in mucus from the frog's mouth or in normal salt solution. Examine with the high power of the microscope. At the free edges of some of the cells a shimmering movement will be seen; this is often so rapid that it is impossible to see the cilia which cause it, but after the movement has slackened somewhat it can be seen to be due to delicate, hair-like processes of the cells. The stroke of a cilium is always stronger in one direction and all the cilia beat in the same direction. *Draw* some of the cells and their cilia.

Ciliated epithelium may also be seen by removing a small piece of the peritoneum supporting the oviduct and mounting it in normal salt solution. An excellent example of ciliated epithelium is afforded by the gills of the fresh-water mussel; mount a small piece (a single layer) of the gill in water.

The following demonstration will be prepared by an instructor: Place a recently-killed frog on its back, cut away the lower jaw and the floor of the mouth in order to expose the roof of the mouth, and place some powdered carmine or bits of cork on the roof of the mouth. Observe that the particles are carried slowly back toward the œsophagus; how?

XIII. The Skeleton.

The skeleton, which forms the hard internal parts of the frog, is composed partly of cartilage and partly of bone. In the early stages of development the skeleton consists entirely of cartilage; in the adult this primary cartilaginous skeleton is replaced to a greater or less extent by bone. Bones formed thus by the replace-

ment of cartilage are called **cartilage bones.** By far the greater portion of the skeleton of the frog is made up of cartilage bones. Cartilage may also be calcified (*i. e.,* become hardened by a deposit of calcareous material) without taking on the histological character of true bone.

Bones may also be developed in places where there is no preexisting cartilage, and are then, in distinction from cartilage bones, called **membrane bones.** Membrane bones are formed as ossifications in the cutis or deeper layer of the skin; in many fishes they retain this primitive position, but in the frog and in most higher vertebrates they sink beneath the skin and come into closer relation to the more deeply placed cartilaginous skeleton. In the frog many of the bones of the skull, and in addition the clavicles, are membrane bones.

With a razor or a sharp scalpel cut off a thin slice of the uncalcified (clear or **hyaline**) cartilage from the proximal end of the femur, mount it on a slide in a drop of water and cover it with a cover glass; examine it first with the low and then with the high power of the microscope. Observe the clear **matrix** and the cell spaces each containing one or more cells. *Sketch* a portion, representing each cell at least one centimeter in diameter.

Study prepared slides showing cross sections of the femur of the frog, verifying so far as possible the description on pp. 126–128 of Holmes' Biology of the Frog. *Sketch* a sufficient portion of the section to show the various structures seen; label all parts. Examine a cross-section of dry bone taken from some mammal; how does it differ in structure from the bone of the frog?

The various parts of the skeleton are either immovably fastened together, or connected in such a way as to allow some freedom of motion. In either case the connection is called an **articulation** or **joint.** Where slight movement is required, the bones are united by pads of tough and elastic fibro-cartilage, as in the joints between the bodies of the vertebræ; in the more movable joints, each articular surface is covered with a pad of cartilage, and the bones are held together by strong bands or capsules of fibrous material, called **ligaments.**

With the aid of the following outline, study both entire and disarticulated skeletons of the frog, and be prepared to demon-

strate all the bones, both in the articulated and disarticulated skeletons.

The skeleton of the frog may be conveniently divided into: (*a*) the **axial skeleton,** including the skull and the vertebral column, and (*b*) the **appendicular skeleton,** including the limbs and the limb girdles which attach them to the body. What evidence of metameric segmentation do you find in the skeleton of the frog?

A. **The Axial Skeleton.**—1. The **vertebral column** consists of nine vertebræ and a posterior unsegmented portion, the **coccyx** or so-called **urostyle.**

(*a*) Select a **typical vertebra,** for example the fourth, for careful study. Observe that it forms a bony ring, enclosing a central **neural canal** which during life is occupied by the spinal cord. The floor of the canal is formed by the **centrum** of the vertebra, which in the complete vertebral column is joined to the centra of adjacent vertebræ by means of pads of fibro-cartilage. The **neural arch** comprises the lateral and dorsal portions of the ring and forms the sides and roof of the neural canal. Projecting dorsally and posteriorly from the top of the neural arch is the **spinous process** or **neural spine.** The **transverse processes** are a pair of large projections extending horizontally outwards from the sides of the neural arch. The two pairs of **articular processes** or **zygapophyses,** situated respectively on the anterior and posterior borders of the neural arches, articulate with corresponding processes of adjacent vertebræ; the **anterior articular processes** or **prezygapophyses** face upward and slightly inward, while the **posterior articular processes** or **postzygapophyses** face downward and slightly outward. *Draw* a posterior view of a vertebra ($\times 4$).

When all its parts are in place the vertebral column forms a more or less complete tube for the protection of the spinal cord, but at the sides of this tube, between the successive vertebræ, are openings, the **intervertebral foramina,** through which nerves pass out from the spinal cord.

(*b*) **Special vertebræ.** The first vertebra or **atlas** articulates with the skull; how? This vertebra has no transverse process; why? In the complete skeleton, observe the large gap on the

dorsal surface between the skull and the neural arch of the atlas. In life this gap is closed by a strong membrane, but it affords a convenient place for beginning dissection to expose the central nervous system, and for dividing and destroying the central nervous system in the operation known as "pithing."

The second to fourth vertebræ have unusually strong transverse processes; why? The ninth vertebra (**sacrum**) has especially strong backwardly directed transverse processes; what for?

(*c*) The **coccyx** or so-called **urostyle** is a long cylinder of bone with a dorsal keel; it articulates in front, by two surfaces, with the body of the sacral vertebra. The neural canal is continued into the anterior portion of the coccyx. On the sides of the coccyx, and about the length of a vertebra from its anterior end, are a pair of small holes through which the tenth pair of spinal nerves pass out; these holes correspond to intervertebral foramina. The entire coccyx represents a row of fused vertebræ (compare the spinal column of a tailed amphibian).

2. The **skull** consists of (*a*) an axial portion, the **cranium,** which encloses the brain; associated with this are two pairs of capsules enclosing sense organs, viz., the **olfactory capsules** forming an anterior prolongation of the cranium, and the **auditory capsules** forming lateral expansions of the posterior region of the cranium; (*b*) the **jaws** and the framework by which they are attached to the cranium, and (*c*) the **hyoid apparatus,** situated mainly in the floor of the mouth.

(*a*) The **cranium.** (1) **Cartilage bones of the cranium.**

The **exoccipitals** are a pair of bones which together bound the **foramen magnum** or posterior opening of the cranium, which is continuous with the neural canal of the spinal column. On their posterior surfaces they bear the **occipital condyles,** two oval convex processes which articulate with the first vertebra or atlas. Near each occipital condyle is a foramen by which the vagus nerve leaves the skull.

The **proötics** are two bones forming the bony part of the auditory capsules and lying laterally and anteriorly to the exoccipitals. Each forms part of the orbit of the eye, and on its antero-ventral face bears a large notch through which pass in life the fifth, sixth and seventh cranial nerves.

The **sphenethmoid** or **girdle-bone** is a bony tube encircling the anterior end of the cranial cavity. In a surface view of the entire cranium this bone is partly concealed by membrane bones.

Ventral and lateral to the proötic is a large depression, the **tympanic cavity** (the middle ear of higher vertebrates), which in the fresh skull is covered laterally by the **tympanic membrane** or **drum membrane** of the ear. At the bottom of the tympanic cavity is an opening, the **fenestra ovalis,** which leads inside the auditory capsule; in the fresh skull the fenestra ovalis is closed by a minute disc of cartilage called the **operculum,** which in turn is joined with the tympanic membrane by a small bony and cartilaginous rod called the **columella.** Like the ossicles of the mammalian ear, these two small structures serve to convey sound waves from the tympanic membrane to the inner ear. In prepared skulls the operculum and columella are often missing.

(2) **Membrane bones of the cranium.**

The **fronto-parietals** are two long flat bones meeting in a median **suture** and forming the roof of the cranium. With what bones do they come in contact?

The **parasphenoid** or **parabasal** is shaped like a dagger lacking the handle; it lies on the ventral side of the cranium.

The **nasals** are two triangular bones on the dorsal surface in front of the sphenethmoid; they are set transversely and a little obliquely, and form the roof of the nasal capsules.

The **vomers** are two bones on the ventral surface of the cranium in front of the sphenethmoid. Each bears a group of teeth, the vomerine teeth.

(3) The **cartilaginous cranium.** In a wet preparation from which the membrane bones have been stripped off, the unsegmented cartilaginous cranium is exposed to view. On its dorsal surface observe the large **anterior** and two small **posterior fontanelles**—window-like openings in the cartilage, closed by membrane only. Observe that the exoccipitals, proötics and sphenethmoid are ossifications in this cartilage. Observe that cartilage also forms the basis of both the olfactory and auditory capsules. Compare the cartilaginous cranium of the frog with the entire cranium of an elasmobranch (*e. g.*, a dog-fish or a skate).

(*b*) **Jaws and suspensorium.** The jaws consist of two carti-

laginous and bony arches, **maxillary** and **mandibular.** A cartilaginous and bony framework, the **suspensorium,** serves to attach the jaws to the cranium.

(1) The **maxillary arch,** or **upper jaw,** contains the following bones in each lateral half:

The **maxilla,** a long thin bone forming the greater part of the upper jaw. It bears teeth along its entire length. Anteriorly it meets the premaxilla, and posteriorly the quadrato-jugal. Describe its relation to the nasal.

The **premaxilla** meets its fellow of the opposite side in the middle line anteriorly. Each bears teeth and gives off from its dorsal surface a backwardly directed process which forms part of the boundary of the nasal opening. In life the premaxillæ are movable and when pressed against by the tip of the lower jaw they are turned slightly backward, closing the nares.

The **quadrato-jugal** is the only cartilage bone of the upper jaw. Its anterior end articulates with the posterior end of the maxilla and its posterior end articulates with the squamosal. It does not bear teeth.

(2) The **suspensorium.** The jaws are attached to the cranium by means of a suspensory apparatus in which the following bones are to be distinguished on each side:

The **tympanic** or **squamosal,** a T-shaped bone forming the postero-lateral wall of the skull. The stem of the T unites with the quadrato-jugal, and the posterior arm of the cross of the T abuts against the lateral wall of the proötic.

The **pterygoid,** a triradiate bone lying beneath the tympanic. What are its relations to other parts of the skull?

The **palatine,** a slender transverse bone lying in the roof of the mouth, and passing between the maxilla and the anterior end of the sphenethmoid.

The nasals, described above, also serve to some extent to brace the maxillae.

Draw ($\times 4$) a dorsal view of the skull; also a ventral view, excluding the lower jaw and the hyoid apparatus.

(3) The **mandibular arch,** or **lower jaw.** Each half consists of a cartilaginous core, Meckel's cartilage (best seen in a wet

preparation), on which are deposited the following membrane bones:

The **angulo-splenial,** a long bone forming the posterior part of the arch; posteriorly it articulates with the maxillary arch. It is grooved on the upper and outer surface for the reception of Meckel's cartilage.

The **dentary,** a thin flat bone lying on the outer surface of the anterior half of Meckel's cartilage.

The mandibular arch is completed in front by two small cartilage bones, the **mento-meckelian** bones; these are ossifications in Meckel's cartilage, on each side of the median line. They are opposed to the premaxillæ, and as previously explained form part of the mechanism for closing the nares.

Compare the mandibular arch of the frog with that of an elasmobranch (*e. g.*, a skate or a dogfish).

(*c*) The **hyoid apparatus.** The main body of the hyoid consists of a cartilaginous plate lying in the floor of the mouth. The following are its more important processes: (1) the **anterior cornua,** slender cartilaginous rods, one on each side, extending backward and upward to their points of attachment to the lower side of the auditory capsules; (2) the **posterior cornua** or **thyrohyoid processes,** which are a pair of stout bony processes diverging from the posterior border of the body of the hyoid; and (3) the **lateral processes,** short cartilaginous projections just in front of the thyrohyoid processes.

Compare the hyoid apparatus of the frog with the more fully developed hyoid and branchial apparatus of an elasmobranch (*e. g.*, a skate or a dogfish). The anterior cornua of the frog correspond to the hyoid arch of the elasmobranch, the lateral and posterior processes are vestiges of branchial arches, while the body of the hyoid represents the fused ventral ends of the hyoid and branchial arches. In the tadpole stage of the frog and the toad, branchial arches are present much as in the adult elasmobranch (see Leuckart's chart).

B. **The Appendicular Skeleton.**—This consists of the bones of the limbs, and the girdles (pectoral and pelvic) which unite them to the axial skeleton. With the exception of the clavicles, all the bones are cartilage bones.

1. The **pectoral girdle** almost completely encircles the body a short distance behind the head. Each lateral half of the pectoral girdle consists of two portions, dorsal and ventral, which meet at the shoulder joint. The dorsal ends of the two halves are attached by ligaments and muscles to the vertebral column, while the ventral ends are united together in the median plane by the sternum or "breast-bone."

(*a*) The **dorsal portion** is made up of two parts:

The **scapula,** a flat elongated bone somewhat constricted in the middle. The postero-ventral angle forms part of the wall of a cup-shaped cavity, the **glenoid fossa,** in which the arm articulates.

The **suprascapula** is a broad plate of cartilage fastened to the dorsal end of the scapula. When in its natural position its thin expanded distal end overlaps the first four vertebræ. In the adult frog, its proximal portion is calcified and more or less ossified.

(*b*) The **ventral portion,** exclusive of the sternum, is formed of two bars:

The posterior bar, much stouter than the anterior, is formed by a single bone, the **coracoid.** Its outer end forms a large part of the glenoid fossa.

The anterior bar is slender and formed of two elements, **clavicle** and **precoracoid,** applied closely together along their entire length. The anterior element, the clavicle, is a membrane bone. The posterior element, the precoracoid, is cartilaginous; in the prepared skeleton it is usually dried so as to be inconspicuous, but its position is indicated by a groove in the posterior side of the clavicle. Its outer end forms a small part of the wall of the glenoid fossa.

Compare the scapula, clavicle and coracoid with these elements in the skeleton of the turtle, where they form a very regular tripod. What is the mechanical advantage of this arrangement of parts? Compare the corresponding parts of the human skeleton.

(*c*) The **sternum** consists of the following parts, beginning at the anterior end:

The **episternum,** composed of a nearly circular plate of cartilage and a bony rod, in front of the median ends of the clavicles.

The paired **epicoracoids** are two cartilages lying between the median ends of the coracoids and precoracoids.

The **sternum proper,** consisting of a rod of cartilage ensheathed in bone, projecting posteriorly from the epicoracoids, and a somewhat bilobed plate of cartilage comprising the posterior end of the sternum.

2. The **fore-limbs.** When the limbs of the frog are extended at right angles to the body with the palms of the hands and the soles of the feet downward, the anterior sides are called **preaxial** and the posterior sides are called **postaxial.** (See Bourne, Comparative Anatomy, vol. 1, fig. 5.) The same side is preaxial or postaxial whatever the position of the limb.

With the exception of the small bones of the wrist and ankle, all the bones of the limbs are elongated, with enlarged ends capped with articular cartilage. The enlarged ends or **epiphyses** ossify independently of the shaft or **diaphysis,** with which they do not unite until late in life. The femur (thigh bone) is a good example of a long bone. Examine a dry femur split lengthwise and observe that it is hollow. What are the mechanical advantages of the cylindrical form of the long bones?

Each anterior limb consists of three parts, **upper-arm, fore-arm,** and **hand.** The proximal part of the hand is distinguished as the **wrist.**

The **upper-arm** or **brachium** contains a single bone called the **humerus.** This is a good example of a long bone. The proximal end or **head** of the humerus rests in the glenoid cavity, forming a ball-and-socket joint, and is held in position by muscles and ligaments The strong ridge on the preaxial side of the proximal portion of the diaphysis is called the **deltoid crest** or ridge, and serves for the attachment of muscles. At the distal end is a spheroidal surface for articulation with the bones of the fore-arm. *Draw* a side view of the humerus ($\times$ 4).

The **fore-arm** contains likewise but a single bone, the **radio-ulna.** This is formed by the fusion of two originally distinct bones (compare the human skeleton). The distal half is imperfectly divided by a groove into preaxial or radial, and postaxial or ulnar, portions. The proximal end is hollowed out to articulate with

the distal end of the humerus, and bears a projection known as the **olecranon process.**

The **wrist** or **carpus** contains six small **carpal** bones arranged in two rows. In the proximal row there are three bones: one corresponding to the radius, the **radiale;** one corresponding to the ulna, the **ulnare;** and one between the two, the **centrale.** In the distal row we find, beginning on the radial or thumb side, two small bones corresponding respectively to the rudimentary thumb and the second digit; these we call **distals 1** and **2.** There is a much larger crescentic bone which represents **distals** 3, 4 and 5 fused together.

The **hand** or **manus** exclusive of the wrist has four complete **digits,** and a rudimentary thumb. Adjoining the carpal bones are five **metacarpals;** the small first metacarpal on the preaxial side represents the thumb (**pollex**). The second and third metacarpals are followed by two **phalanges** each, the fourth and fifth by three each. The phalanges form the fingers.

Draw ($\times 4$) the manus, including the wrist.

3. The **pelvic girdle** or **pelvis** as a whole is V-shaped. In the adult frog the girdle is placed very obliquely, so as to be nearly parallel to the vertebral column instead of at right angles to it. The two limbs of the V diverge anteriorly and are fastened to the strong backwardly directed transverse processes of the ninth or sacral vertebra. Each lateral half of the pelvic girdle is composed of three parts, which meet in the **acetabulum,** a rounded cavity which receives the head of the femur and forms with it the hip-joint. The three parts are:

The **ilium,** the long bone forming a limb of the V. Observe on its dorsal surface a prominent ridge, the **iliac crest.** The ilium is attached in front to the tip of the transverse process of the ninth vertebrae; posteriorly it forms the anterior and dorsal half of the acetabulum. Posteriorly the two ilia meet and are united in the median plane to form the **iliac symphysis.**

The **ischium** forms the postero-dorsal portion of the pelvis and the posterior third of the acetabulum. The ischium is roughly oval in form, with a dorsal prominence. The two ischia are united by the whole of their median surfaces, forming the **ischial symphysis.**

The **pubis** is a triangular plate of cartilage, more or less calcified, wedged in between the ilium and ischium ventrally and forming about one-sixth of the acetabulum. The two pubes are united by the whole of their median surfaces, forming the **pubic symphysis.**

Draw (× 4) the pelvic girdle viewed from the lateral aspect.

Compare the pelvic girdle and its relation to the vertebral column in the frog with the corresponding features in a tailed amphibian (urodele). In the phylogenetic history of the frog the fusion of the posterior vertebrae to form the coccyx was doubtless associated with a forward migration of the attachment of the pelvic girdle, and the elongation of the ilia. The urodele presents the more primitive condition, and the peculiar structure of this part of the skeleton of the frog is the result of specialization correlated with its leaping habits.

Examine the pelvic girdle in skeletons of the turtle and of man.

Obviously the pectoral and the pelvic girdles are built on essentially the same plan. Structures that are metamerically repeated are said to be **serially homologous.** What element in the pectoral girdle is homologous with each of the elements of the pelvic girdle? Better examples of serially homologous structures are found in the vertebral column, and in the limbs.

4. The **hind-limb.** The bones have the same general character as those of the fore-limb, with which they are homologous.

The **thigh.** The **femur** or thigh-bone is a long bone with a slight sigmoid curve. The proximal end or **head** is spheroidal and fits into the acetabulum, forming the hip-joint; the distal end is somewhat broadened for articulation with the tibio-fibula.

The **leg** or **crus.** The **tibio-fibula** is slightly longer than the femur, slightly curved, and broadened at both ends. Like the radio-ulna, this is a compound bone, formed of two bones usually separate in other animals (compare the human skeleton). Near each end, a longitudinal groove indicates the line of fusion of the two bones.

The **ankle** or **tarsus** contains five **tarsal** bones. The two proximal ones are long bones united at each end by common epiphyses. The preaxial one is known as the **tibiale** or **astragalus,** the postaxial and larger one is the **fibulare** or **calcaneum.**

The **centrale** is represented by a small bone—a mere nodule—on the preaxial side of the distal end of the tibiale; it supports the prehallux (see below). Between the two proximal tarsal bones and the metatarsals are two very small irregular tarsal bones corresponding to the distal row of carpal bones in the wrist. Of what advantage to the frog is the elongation of the two proximal tarsal bones?

The **foot** or **pes** exclusive of the tarsus has five complete digits and a small supernumerary digit. The supernumerary digit is on the preaxial side of the foot and consists of from one to three small bones; it is sometimes called the **prehallux.** The first of the long digits on the preaxial side corresponds to the great toe of man, and is called the **hallux.** Each of the long digits consists of a **metatarsal** bone, and **phalanges** forming the toes as follows: the first and second toes have two phalanges each, the third three, the fourth four, and the fifth three.

Draw ($\times$ 4) the foot including the ankle.

Make a *table* showing the homologies between the elements of the fore-limbs and the hind-limbs.

XIV. The Muscular System.

The movements of the frog are effected by means of muscles. The distinguishing property of muscular tissue is a high degree of **contractility:** *i. e.*, the muscle fiber shortens, thereby bringing the two ends, and consequently the parts to which it is attached, nearer together.

A. **The Finer Structure of the Muscular System.**—Muscular tissue may be classified according to the structure of its cells into two kinds, **striated** and **non-striated.** The former presents two varieties: (*a*) a kind forming the skeletal muscles, which consist of unbranched striated cells each containing many nuclei; (*b*) another kind, found in the heart, is called **cardiac muscular tissue,** and consists of striated cells which branch, and contain each a single nucleus. The non-striated muscle cells are usually unbranched, are lacking in cross-striations, and contain each a single nucleus.

With regard to nervous control, muscular tissue may be classified as **voluntary** and **involuntary.** The voluntary muscle fibers

are under the immediate control of the central nervous system; this class includes practically all the skeletal muscles. The involuntary muscle fibers are more or less under the control of the sympathetic nervous system; this class includes both the non-striated muscle fibers and the cardiac muscular tissue.

1. **Non-striated muscle fibers.** These have already been encountered in the study of cross-sections of the stomach and intestine. They may be studied to better advantage in stained and mounted microscopical preparations of the wall of the bladder. In such a preparation, observe that the fibers are usually grouped in bundles; the more isolated fibers are better for study. Make out if possible the outlines of a single fiber; observe that it has the form of a very slender spindle, usually unbranched but in rare cases forked at one end or even at both ends. In its center lies a single elongated nucleus, and at each end of this nucleus is some granular cytoplasm. The fibers sometimes exhibit a faint longitudinal striation. Under high power, *draw* a non-striated muscle fiber.

2. **Striated muscle fibers.** Only the fibers of the skeletal muscles will be considered here. Cut out a small piece of such a muscle from a freshly-killed frog; mount it on a slide in a drop of normal salt solution[1] and tease the fibers apart with dissecting needles. Cover with a cover glass and examine with the low and then with the high power. Observe the very long fibers or muscle cells, with their transverse striations consisting of alternate light and dark bands. There are also longitudinal striations, which divide the fiber into **fibrillæ.** Observe the **sarcolemma** or cell-wall of the fiber; this is so delicate as to be visible only in crushed or twisted fibers. The **nuclei** are best seen after running a little dilute acetic acid (3 per cent.) under the cover glass; this is best done by placing a drop of the acetic acid on the slide in contact with one edge of the cover glass, and drawing out the liquid from the opposite edge by means of a piece of absorbent paper. Observe that there are many nuclei, and that they are elongated and highly refractive. Each nucleus

[1] A physiologically normal salt solution is one whose osmotic pressure with respect to the tissue cells is equal to that of the plasma of the blood This solution is made by adding 7 grams of sodium chlorid to one liter of water.

lies in an undifferentiated portion of the protoplasm. *Draw* a portion of a fiber, showing details of structure, representing the fiber about 25 mm. in diameter.

B. **General Anatomy of the Skeletal Muscles.**—A skeletal muscle consists of a bundle of striated muscle fibers bound together by connective tissue and fastened at each end to different bones, by means of strong cords called **tendons.** Of the two attachements of a muscle, one is usually to a more fixed and central part, the other to a more movable and peripheral part; the former attachment is called the **origin** of the muscle, the latter its **insertion.**

Since muscles can exert force only while shortening and not while elongating (in other words, since they can pull but cannot push), many of the muscles or sets of muscles are arranged in pairs, the members of a pair working in apposition to each other.

The many actions of which the frog is capable require a large number of separate muscles, only a few of which can be considered here. To illustrate the mode of attachment, functions and mutual relationships of the skeletal muscles a study will be made of the principal muscles of the hind limbs.

The motions of which a limb is capable are conditioned, not only by the number and arrangement of its muscles, but by the form of its joints, some of which allow only certain movements. In the hind limb the ball-and-socket joint at the head of the femur allows great freedom of motion, but other joints permit of movement approximately in only one plane.

With respect to the kind of motion produced the muscles of the limbs of the frog may be classified as **abductors, adductors, rotators, flexors,** and **extensors.** When the thigh of the frog is pulled forward (*i. e.,* away from the median line), it is said to be abducted; when it is pulled backward (*i. e.,* toward the median line), it is said to be adducted. When a muscle of the thigh is placed obliquely its effect is generally to rotate the thigh on its long axis. When the leg (**crus**) of the frog is bent so as to form an angle with the thigh, the leg is said to be flexed; when it is brought in line with the thigh, the leg is said to be extended. The leg is incapable of rotation. The foot may be flexed, extended, or slightly rotated.

The "ventral" and "dorsal" surfaces of the hind leg are such in consequence of the extreme obliquity of the pelvic girdle; they are really preaxial and postaxial, and correspond respectively to the inner and outer surfaces of the human leg.

The muscles should be studied in a frog that has been hardened in formalin. Aid in the identification of the muscles may be obtained by consulting figure 70 of Holmes' Biology of the Frog, or figure 11 of Marshall's The Frog. The origin and insertion of each muscle should be carefully determined, and then located on the skeleton of the frog. The action of the muscle may sometimes be tested by pulling it gently, but this is often impracticable in preserved material; the action of a muscle may generally be inferred from its position. For the more accurate determination of the action of a muscle, stimulation by an electric current in the freshly-killed frog is required.

Construct a table giving the origin, insertion and action of each of the muscles named below. In the description of the action of a muscle, it should be stated what movement the muscle would accomplish if it worked alone; the effect of the contraction of a muscle is sometimes different when it acts in coöperation with other muscles. Having completed your table compare it with the description in Holmes' Biology of the Frog, correct your own if wrong, and then verify the corrections on your specimen.

In removing the skin from the hind limb of the frog, be careful not to remove with it the thin muscle which is attached to the skin along the posterior side of the thigh. The dissection of the muscles will consist mainly of separating them by means of a seeker. In case it becomes necessary to cut a muscle in order to expose the muscles underneath, this should be done by dividing it transversely in the middle and reflecting the two halves, leaving the origin and the insertion intact.

1. The **thigh.** (*a*) **Superficial muscles of the preaxial side.** This surface is ventral when the thigh is adducted and the leg extended.

The **sartorius,** a narrow thin muscle which extends somewhat obliquely from the abductor surface of the thigh at its proximal end to the adductor surface at its distal end.

The **adductor magnus,** a large muscle on the adductor side of

the sartorius; distally it passes beneath the sartorius. About its middle it receives a small slip which originates on the tendon of one of the heads of the semitendinosus; this will be examined later.

The **adductor longus** is a narrow thin muscle lying along the abductor side of the sartorius, and more or less hidden by it. Divide the sartorius about its middle and reflect its parts; this will expose the adductor longus, whose fibers mingle with those of the adductor magnus along its distal third.

The **gracilis major** or **rectus internus major,** a powerful muscle on the adductor side of the adductor magnus. About its middle it is crossed by an oblique **tendinous inscription** or **tendinous intersection.** (The tendinous inscriptions of the limb muscles are homologous with those of the abdomen, which are segmentally arranged—an inheritance from the metameric condition of the body muscles in the fishes and the tailed amphibians.)

The **gracilis minor** or **rectus internus minor** is a narrow flat muscle running along the adductor surface of the thigh and partly covering the gracilis major. It is sometimes removed with the skin.

(*b*) **Superficial muscles of the postaxial side.** This side is dorsal when the thigh is adducted and the leg extended.

The **triceps femoris,** a very large muscle occupying the entire abductor surface of the thigh. It originates by three heads, anterior, middle and posterior; the anterior head lies partly on the preaxial surface.

The **semimembranosus,** a large muscle lying along the adductor surface. There is an oblique tendinous inscription running along this muscle, as in the gracilis major.

The **ileo-fibularis,** a slender muscle lying between the triceps femoris and the semimembranosus.

The **iliacus externus** arises far up on the crest of the ilium and lies between the anterior and middle heads of the triceps femoris.

The **pyriformis** is a slender muscle passing between the origins of the semimembranosus and the posterior head of the triceps.

(*c*) The **deep muscles of the thigh** will be omitted with the exception of the **semitendinosus,** a long slender muscle lying be-

tween the adductor magnus and the gracilis major. It arises by two heads; the anterior head passes through a slit in the adductor magnus and unites with the posterior head in the distal third of the thigh; its tendon of origin gives attachment to a small slip which belongs to the adductor magnus.

2. The **leg (crus).** The **gastrocnemius** lies along the flexor surface of the tibio-fibula, forming the calf of the leg. Its origin is by two heads; distally the muscle ends in the strong tendon of Achilles. Divide and reflect the gastrocnemius.

The **tibialis posticus** lies between the gastrocnemius and the tibio-fibula.

The **tibialis anticus longus** lies along the extensor surface of the tibio-fibula. It is a slender muscle which divides posteriorly into two slips.

The **peroneus** lies on the postaxial side of the leg, between the tibialis anticus longus and the gastrocnemius.

The **extensor cruris** is a small muscle lying on the preaxial side of the anterior half of the tibialis anticus longus. Divide and reflect the latter; this will expose:

The **tibialis anticus brevis.** The body of this muscle lies immediately beneath the preaxial slip of the tibialis anticus longus.

XV. Connective Tissue.

Connective tissue serves to unite the various elements of an organ, binds together muscle fibers, and forms tendons and ligaments. There are several varieties of connective tissue, which agree in that the intercellular substance is large in amount. Examples of connective tissue have already been encountered in the study of cross-sections of various organs, particularly the alimentary canal and the skin. For more careful study connective tissue should be prepared as follows: From a preserved specimen cut out a small piece of the white fibrous connective tissue which binds the skin to the body, spread it out on a slide in a drop of water, and cover it with a cover glass. Under high power observe the numerous fibers, which are usually unbranched and of a characteristic wavy appearance. Irregularly distributed among the fibers are the **connective tissue corpuscles** or cells; these have conspicuous granular nuclei and vary considerably in

form and in the appearance of the cytoplasm. *Sketch* a small portion of connective tissue.

XVI. The Nervous System.

A. **General Anatomy.**—The nervous system is composed of two great divisions, the **central** and the **peripheral.** The central nervous system includes the brain and the spinal cord; the peripheral system includes all the nervous structures outside the cord: the cranial, spinal and sympathetic nerves, and various small ganglia. The system of nerves and ganglia which supplies the viscera is sometimes considered separately as the **sympathetic nervous system,** and in distinction from it all the rest of the nervous system is called the **cerebro-spinal nervous system.**

1. **The central nervous system.** The method of making the following dissection will be demonstrated at each table, but each student should do the work on his own specimen. Expose the brain and spinal cord as follows: Remove the skin from the dorsal surface of the body, and dissect away the muscles which cover the vertebræ; then cut through the tough membrane roofing over that portion of the brain between the skull and the first vertebra, taking care not to injure the brain. Through the opening thus made introduce one blade of a pair of strong scissors and cut away the roof of the cranium. This is best done by making a forward incision through the roof of the cranium on each side of the brain, then with forceps removing the piece between these two cuts. Working backward from the same starting-point, in like manner remove the roof of the spinal canal, in which the cord lies, by cutting through the neural arches of the vertebræ.

Observe that the cord possesses two enlargements, the **anterior** or **brachial** and the **posterior** or **lumbar** enlargement. Behind the lumbar enlargement the cord gradually tapers to a point. Observe the **dorsal longitudinal fissure.** Carefully cut down the stumps of the neural arches and expose the **roots of the spinal nerves.** As these roots pass through the **intervertebral foramina** they are surrounded by the dense, white **calciferous bodies** or so-called **periganglionic glands,** each of which, as the latter name implies, encloses a spinal ganglion. The calciferous bodies are not glandular in nature, but represent extensions of the

endolymph space of the outer covering membrane (**dura mater**) of the spinal cord, filled with fine granules of calcium carbonate.

Beginning at the posterior end, the divisions of the brain as viewed from the dorsal aspect are:

(*a*) The **medulla oblongata.** This may be easily recognized by the triangular cavity on its dorsal surface. This cavity is the **fourth ventricle** of the brain and is continuous with other cavities (**ventricles**) in the brain and with a **central canal** in the cord. The cavity of the fourth ventricle is roofed over by vascular epithelium which is thrown into numerous inturned folds forming the **posterior choroid plexus.**

(*b*) The **cerebellum** is the transverse fold lying in front of the medulla. In the frog it is very small.

(*c*) The **optic lobes** are two rounded masses immediately in front of the cerebellum.

(*d*) The **thalamencephalon** is the portion between the optic lobes and the cerebral hemispheres (see below). Its cavity is known as the **third ventricle;** the roof of the latter is very thin and vascular and forms the **anterior choroid plexus.** Near the center of this arises a slender lobe, the **pineal body** or **epiphysis,** which extends forward over the thalamencephalon; a small **parietal nerve** runs along the dorsal surface of the epiphysis and passes through the skull to the brow spot. Both epiphysis and parietal nerve are frequently torn away from the brain when the roof of the cranium is removed. In front of the epiphysis and between the posterior ends of the cerebral hemispheres is the **paraphysis,** a vascular and probably glandular outpocketing of the roof of the thalamencephalon.

(*e*) The **cerebral hemispheres** are two anterior swellings separated from the forward-projecting **olfactory lobes** by a slight constriction. The olfactory lobes are continued forward as the **olfactory nerves** (first pair of cranial nerves).

Draw a dorsal view of the central nervous system, twice natural size. Make this drawing in the center of a clean page, so that other features may be added later.

After studying the peripheral nervous system (see below), remove the brain and spinal cord along with the roots of all the nerves so far as possible. Begin at the anterior end; cut through

the olfactory nerves, then turn the brain back, cutting through the nerves as they come into view. Great care is necessary to avoid tearing off the exceedingly delicate cranial nerves. Immerse the nervous system, ventral surface up, in water, and observe:

(*a*) The **ventral longitudinal fissure** of the spinal cord.

(*b*) On the ventral side of the thalamencephalon the **optic chiasma** formed by the crossing of the **optic nerves** (second pair of cranial nerves); the slightly bi-lobed **infundibulum** posterior to the chiasma; posteriorly the infundibulum is continuous with the **pituitary body** or **hypophysis** which is generally torn off when the brain is removed from the cranium.

Compare the brain of the frog with figures or a model of the brain of man. What parts are relatively larger in the human brain?

With a sharp scalpel, razor, or scissors carefully slice off the roof (dorsal wall) of the brain until the cavities are exposed throughout its entire extent. In the cerebral hemispheres make out a pair of cavities, the **first** and **second ventricles;** do they extend into the olfactory lobes? The unpaired cavity (**third ventricle**) of the thalamencephalon connects in front with the first and second by transverse passages, the **foramina of Monro.** In the optic lobes are a pair of **optic ventricles,** which, since they do not occur in mammals, are not numbered. The optic ventricles communicate with a median cavity, the **aqueduct of Sylvius** or **iter e tertio ad quartum ventriculum,** which, as the latter name implies, connects the third ventricle with the **fourth ventricle** situated in the medulla.

Study prepared slides showing cross-sections through different regions of the brain. With the aid of figures showing sagittal and horizontal sections (see Holmes' Biology of the Frog, figures 83 and 84), determine from what parts of the brain the cross-sections are taken. Make outline *drawings* of sections through four different regions, and label all the parts shown.

2. The **peripheral nervous system.** Of the cranial nerves, the olfactory and optic nerves are readily observed during the study of the brain. On account of the difficulty attending their dissection in the frog, the smaller cranial nerves are omitted; they

may be studied to much better advantage in an elasmobranch (skate or dog-fish). Only the spinal nerves will be considered here.

The **spinal nerves** are segmentally arranged, and are given off in pairs on each side of the spinal cord. Each spinal nerve arises by two roots, a **dorsal** and a **ventral.** The dorsal root is known as the **sensory** root because it conducts nerve impulses from the sense organs to the cord (**afferent** impulses), and the ventral root is known as the **motor** root because it conducts nerve impulses outward (**efferent** impulses) to the muscles. Just outside the spinal canal the two roots unite to form a common trunk; a small spinal ganglion lying in the intervertebral foramen and surrounded by the white calciferous body or so-called periganglionic gland is found on the dorsal root near its union with the ventral root, but as a rule the spinal ganglion is continued beyond the point of the union so that the two roots appear to meet in the ganglion.

The distribution of the spinal nerves is best studied by examining the *inner* surface of the dorsal body wall; on each side of the vertebral column the paired spinal nerves will be found exposed throughout a considerable part of their course. Before beginning the study of the cranial nerves, identify the sympathetic system (see below) in order to avoid injuring it.

To expose the first and second spinal nerves, turn the roof of the oesophagus forward and cut it off close to the mouth cavity.

(*a*) The **first spinal** or **hypoglossal.** This nerve leaves the spinal canal between the first and second vertebræ; it passes around the side of the throat, turns forward below and behind the angle of the jaw and ends in muscles of the floor of the mouth.

(*b*) The **second spinal** or **brachial** is the largest spinal nerve. It leaves the vertebral canal between the second and third vertebræ, and passes outward to supply branches to the shoulder region and arm. Why is it so large? The second spinal is connected with the third by one or more delicate cross-branches; usually it is also connected with the first by other very slender twigs. The nerves of this region where the cross-connections

occur are sometimes said to constitute a **brachial plexus.** There is considerable variation in the cross-branching in different specimens of the frog, but the brachial plexus is never so well developed as in the mammals where it takes the form of a complicated network.

(*c*) The **third spinal** nerve is small and emerges from between the third and fourth vertebræ; for some distance it runs along the posterior edge of the brachial nerve with which it is connected by one or more slender branches, and finally supplies some muscles of the body wall.

(*d*) The **fourth, fifth and sixth spinal** nerves are small and run obliquely backward. Determine their distribution.

(*e*) The **seventh, eighth and ninth spinal** nerves pass almost directly backward and anastomose with each other to form the **sciatic plexus.** The eighth and ninth unite to form the large **sciatic** nerve which may be followed by separating the great muscles of the dorsal surface of the leg.

(*f*) The **tenth** pair of spinal nerves are very small and pass almost directly backward. They innervate the bladder and the cloaca.

Add the spinal nerves to your *drawing* of the central nervous system; represent them twice natural size.

3. The **sympathetic system** centers in two delicate chains of ganglia extending longitudinally, one on each side of the body, lying close to the dorsal aorta and systemic arches. Turn the viscera to one side and lift up the kidney; this will expose the sympathetic system of one side. A nerve trunk unites the ganglia of each chain. About how many ganglia are there? Are the sympathetic ganglia united with the spinal nerves? If so, how? The sympathetic system innervates the internal organs generally. Branches of the sympathetic unite to form plexuses, the largest of which, the **solar plexus,** sends branches to the stomach and adjacent organs.

Add the main trunks of the sympathetic system to your *drawing* of the central nervous system; show also the connections between the sympathetic system and the spinal nerves.

B. **The Finer Structure of the Nervous System.**—Nervous tissue is made up of two different elements: (*a*) the nervous ele-

ments proper, called **nerve cells, ganglion cells** or **neurons,** and (*b*) **neuroglia cells** and connective tissue, whose function is to support and bind the nerve cells together.

A nerve cell or neuron consists of a compact or angular **cell body,** containing the nucleus, and numerous slender processes of which one or more may be very long and are distinguished as **axons,** or **nerve fibers.** The essential part of an axon or nerve fiber is the **axis cylinder,** and in some cases this is the only part present; but usually the axis cylinder is enclosed in two additional layers, described below, which are not properly parts of the nerve cell but are added to the axis cylinder from without.

1. **Nerve fibers.** Cut out a piece of nerve from a freshly-killed frog and with needles tease it apart in normal salt solution. The nerve will be seen to consist of a large number of straight, unbranched nerve fibers, bound together by connective tissue. Examine with the high power. In a fiber make out:

(*a*) The **axis cylinder,** forming an apparently homogeneous central axis.

(*b*) The **medullary sheath,** or **white substance of Schwann,** forming a thick, highly refracting coat around the axis cylinder. The medullary sheath is composed largely of a fatty substance called **myelin,** which often becomes aggregated into irregular masses.

(*c*) The **neurilemma,** or **sheath of Schwann,** a very thin membrane outside the white substance. It is best seen at the nodes (described below).

(*d*) **Nodes of Ranvier,** constrictions where the medullary sheath is interrupted. The segments of the nerve between the nodes are called **internodes.** The nodes occur only at considerable intervals.

(*e*) **The incisures of Schmidt,** oblique markings at rather close intervals, across the medullary sheath. These are sometimes difficult to make out.

(*f*) In each internode is a **nucleus,** lying just beneath the neurilemma and surrounded by a small amount of cytoplasm.

In the development of a nerve fiber the axis cylinder appears first; as it grows out from the cell body, making its way through the other tissues, it becomes surrounded by nucleated cells which

flatten out and form the neurilemma; the white substance appears at a comparatively late period.

Draw a portion of a nerve fiber, showing as many as possible of the points described above. The nerve fiber should be represented at least one centimeter in diameter.

2. **Cross-section of the spinal cord.** Observe the median **dorsal** and **ventral longitudinal fissures.** The ventral margin may usually be distinguished from the dorsal by means of an artery that runs along the ventral longitudinal fissure. Sometimes this artery is removed in making the preparation; in this case the ventral margin must be determined by observing the positions of the ventral white commissure and the larger cell bodies (see below). Observe the **central canal** with its epithelial lining (**ependyma**). The central portion of the cord is composed of **gray matter** consisting mainly of nerve cells (or more strictly speaking the cell bodies of nerve cells) and nerve fibers; the outer portion or **white matter** contains no cell bodies of nerve cells but consists chiefly of fibers which extend in a longitudinal direction. With respect to the distribution of structures on each side of the dorso-ventral median line, the cross-section of the cord shows marked bilateral symmetry.

Many fibers cross over from one side of the cord to the other. Ventral to the gray matter is a rather conspicuous pair of bundles of fibers crossing each other at various angles, forming the **ventral white commissure.** A narrow median tract of less conspicuous fibers crossing in the gray matter above the central canal forms the **dorsal gray commissure;** a similar tract of fibers crossing in the gray matter below the central canal forms the **ventral gray commissure.**

In the **ventral cornua** or horns of gray matter (portions of the gray matter situated ventro-laterally) may be seen a few cell-bodies of nerve cells of unusually large size; processes from these cell-bodies go to form fibers of the ventral or motor roots of the spinal nerves. The fibers of the dorsal or sensory root have their origin in the ganglion of this root, previously described.

In the white matter the longitudinal nerve fibers are, of course, cut transversely. Examine the cut ends of these fibers under

high power; the axis cylinder of each fiber appears as a dark spot surrounded by a clear space occupied by the medullary substance.

The nervous elements of the cord are bound together by stellate neuroglia cells and by processes which arise from the tapering outer ends of the ependyma cells; these processes branch repeatedly and some of them extend to the periphery of the cord. Occasional capillaries may appear in the sections.

The nerve cord is closely surrounded by the following coverings: (*a*) an outer firm membrane, comprising the *inner* layer of the **dura mater;** and (*b*) an inner vascular membrane, corresponding to both the **arachnoid** and the **pia mater** of human anatomy. Both membranes are sometimes torn away in making the preparation.

Under low power, *draw* a cross-section of the cord. The drawing should be about 12 centimeters in diameter.

Be sure you understand the paths into and through the cord of an impulse involved in a reflex act (see below).

C. **Reflex Action.**—This experiment will be performed by an instructor. Kill a frog by cutting across the spinal cord between the skull and the first vertebra; then cut off the entire head except the lower jaw. Suspend the animal by the lower jaw so that the hind legs hang free. Pinch one of the toes and note that the leg is withdrawn. The nerve impulse set up by the irritation of the foot travels along sensory nerve fibers (*i. e.*, fibers which terminate in sense organs) to the cord where it starts a motor impulse along the nerves innervating the muscles of the leg, causing them to contract. Such a direct response to the stimulus is called reflex action. Touch the foot with a hot needle, or with a bit of absorbent paper moistened with dilute acid, and observe the result. Apply dilute acid to the side of the body and see if the foot is used to wipe the acid away. Cut open the body cavity of the frog and lay bare the large nerves going to the hind legs. Irritate one of these nerves and observe the result. Cut one of these nerves across, irritate the end that is in connection with the leg, and observe the effect.

XVII. The Eye.

Review your observations on the external anatomy of the eye of a living frog (Section II, 1). The following work may be done on a preserved specimen, though a freshly killed specimen is better.

Remove an eye-ball by cutting around it through the lids, and severing the muscles and the optic nerve. The muscles that move the eye-ball will not be considered here, since they may be studied to better advantage in a larger vertebrate (*e. g.*, an elasmobranch or a mammal). Observe how the lids are connected with the eye-ball by means of a membrane called the **conjunctiva;** clean away the fragments of the lids from the eye-ball.

Observe carefully the shape of the eye-ball. In the living frog it is nearly spherical but slightly top-shaped; in preserved material it may be slightly flattened on the outer side where it is covered by the cornea. Make an *outline* (8 cm. in diameter) showing the shape of the eye-ball as viewed from its side (*i. e.*, viewed at right angles to the axis passing from the center of the pupil through the center of the eye). This outline is later to be filled in to show the structure of the eye.

The **sclerotic** is the firm outer wall of the eye, composed of hyaline cartilage and dense white connective tissue; it covers all that part of the eye-ball not covered by the cornea. The **optic nerve** may be seen piercing the sclerotic to enter the eye-ball on its proximal side.

With scissors divide the eye into two unequal parts by an incision passing a little to one side of the pupil and the optic nerve, so as to lay open completely the interior of the eye. Observe the sudden collapse of the eye-ball as soon as its fluid contents are allowed to escape. Remove the other eye-ball and divide it into two parts by an incision separating proximal and distal halves. Place the parts of both eye-balls in water and observe the following features:

1. The **crystalline lens** is a solid body, transparent in life, situated just behind the iris and attached to the iris by its outer margin. It is more convex on its inner than on its outer surface.

2. The **anterior chamber** of the eye is the space between the

cornea and the lens; it is small, and in life contains a watery fluid, the **aqueous humor.**

3. The **posterior chamber** of the eye is a large space behind the lens; in life it is filled with a grayish gelatinous body, the **vitreous humor.** In preserved material the vitreous humor may not be readily apparent: it may be shrunken and fused indistinguishably with the retina.

4. The **choroid membrane** is the black pigmented layer lining the sclerotic and continuous in front with the iris. What is its function? Why is the pupil of the eye of the living frog black?

5. The **retina** is a delicate membrane whose sensitive portion lines the eye-ball within the choroid membrane, and is thickest in the proximal region of the eye-ball. In life the sensitive portion of the retina is nearly transparent, slightly grayish; in preserved material it is yellowish-white. In fresh material, the retina is marked on its inner surface by a distinct white spot, the **"blind spot,"** at the point where the optic nerve enters. The retina is readily detached from the choroid, except at the point where the optic nerve enters.

Using the outline already made, construct a *diagram* showing the structure of the frog's eye in a proximo-distal section passing through the pupil and the optic nerve. Label all the parts.

XVIII. The Ear.

Cut off the head of a frog (the specimen used for general dissection will do) just posterior to the tympanum, and decalcify the skull by immersing the entire head for several days in 50 per cent. alcohol containing 10 per cent. nitric acid. Remove the skin and observe the **tympanic membrane** or **drum membrane** of the ear (transparent after the removal of its outer or tegumentary layer), supported at its edges by a cartilaginous ring. To the center of the inner surface of the drum membrane is attached the distal end of a small bony and cartilaginous rod, the **columella,** visible through the tympanic membrane. Tear off the tympanic membrane without disturbing the columella, and observe a large cavity, the **tympanic cavity** (corresponding to the middle ear of man and other mammals), which communicates with the mouth cavity by means of the Eustachian tube. Clean away the muscles

surrounding the auditory capsule. Observe precisely where the proximal end of the columella is attached; here it articulates with a small disc of cartilage, the **operculum,** which fills a small aperture, the **fenestra ovalis,** leading from the tympanic cavity into the cavity of the auditory capsule (**inner ear**). With a sharp scalpel carefully slice off the roof of the auditory capsule in order to expose the inner ear and observe its relation to the operculum. Like the ossicles of the mammalian ear, the columella and operculum serve to convey sound waves from the tympanic membrane to the inner ear.

Observe within the auditory capsule a partially transparent, slightly pigmented sac, the **membranous labyrinth.** With the aid of figure 93 of Holmes' Biology of the Frog make out the following parts: (*a*) an upper part, the **utriculus,** which gives off (*b*) the three **semicircular canals,** lying at right angles to each other and occupying the three dimensions of space; and (*c*) a smaller inferior part, the **sacculus.** For the probable functions of these parts consult the text of Holmes' Biology of the Frog. Find the **auditory nerve;** this is the eighth cranial nerve.

With a dissecting lens and with the low power of the microscope study prepared slides showing stained serial cross-sections taken through the auditory region of the head of a small frog.

Make a *diagram* showing the relations of the various parts of the ear of the frog.

APPENDIX.

I. Text and Reference Books.

The following illustrated works are the most generally useful:

Bourne, Gilbert C. 1909. An Introduction to the Comparative Anatomy of Animals. Vol. I. George Bell and Sons, London.

Ecker, A. 1889. Anatomy of the Frog. Translated by George Haslam. Clarendon Press, Oxford.

Ecker und Wiedersheim. 1896–1904. Anatomie des Frosches, auf Grund eigener Untersuchungen durchaus neu bearbeitet von Dr. Ernst Gaupp. Braunschweig, Friedrich Vieweg und Sohn.

Gadow, Hans. 1901. Amphibia and Reptiles. Vol. 8 of the Cambridge Natural History. The Macmillan Co., New York.

Holmes, S. J. 1907. The Biology of the Frog. The Macmillan Co., New York.

Marshall, A. Milnes. 1896. The Frog: An Introduction to Anatomy, Histology and Embryology. The Macmillan Co., New York.

Mivart, St. George. 1881. The Common Frog. The Macmillan Co., New York.

II. Purchase of Material and Equipment.

Rana pipiens is the species of frog usually furnished by dealers, under the name "grass frog" or "leopard frog." The larger the frogs the better, especially in case the arteries are to be injected. A few large bullfrogs (*Rana catesbiana*) are valuable for permanent preparations, but the supply is uncertain. Material and teaching accessories may be obtained from the following sources:

A. A. Sphung, North Judson, Indiana. (Live frogs.)

E. R. Neuenfeldt, 225 North Clark St., Chicago. (Live frogs.)

Supply Department, Marine Biological Laboratory, Woods Hole, Mass. (Preserved frogs, including specimens with arteries injected.)

F. D. Lambert, Tufts College, Mass. (Living and preserved frogs; arteries injected.)

Brimley Bros., Raleigh, N. C. (Preserved frogs.)

Ward's Natural Science Establishment, Rochester, N. Y. (Preserved frogs; anatomical preparations and models.)

Kny-Scheerer Co., 225 Fourth Ave., New York. (Preserved frogs; anatomical preparations and models; syringe for injecting arteries; dissecting instruments; charts.)

Ernst Leitz, 30 E. 18th St., New York. (Microscopes and accessories.)

Bausch & Lomb Optical Co., Rochester, N. Y. (Microscopes and accessories; dissecting instruments.)

Spencer Lens Co., Buffalo, N. Y. (Microscopes and accessories; dissecting instruments.)

III. Care of Living Material.

Live frogs may be kept in a tank containing one or two inches of water. Frogs in captivity often die from the attacks of parasites; loss from this source may be minimized by frequently cleaning the tank and changing the water, also by keeping only a small number of frogs in a single tank. If kept in a cool place, frogs do not require food during the winter season.

In the laboratory a few frogs may be kept for observation and experiment in small cages supplied with water. Insect cages are excellent for this purpose; they should open at the top. For studying the swimming and diving movements a few small frogs may be placed in battery jars nearly full of water; if the water is warm the frogs will not stay long at the bottom. For individual work on the living frog each student should be supplied with a specimen covered with a finger-bowl inverted over a glass plate (one frog is sufficient for two students). The students should be cautioned not to allow the frogs to escape; for studying the leaping movements use the frogs in the cages.

IV. Killing and Preserving.

As a rule it is best to kill the frogs with sulphuric ether or with chloroform. Ether has some advantages over chloroform in that it leaves the specimens limp instead of rigid, and to most persons the odor of ether is not so unpleasant as that of chloroform. Place the frogs in an air-tight glass jar containing a liberal quantity of cotton saturated with the anæsthetic. In case it is desired to dissect the freshly-killed specimens, the frogs should be anæsthetized at least one hour before they are needed; in such frogs, after a time the heart will usually resume its beating, as is desirable. If the frogs are anæsthetized for a shorter time they may become active during dissection, a circumstance which is very disconcerting to both student and teacher. If it is not desired to demonstrate the action of the heart, the frogs may be

killed by drowning or asphyxiation in an air-tight jar, but this requires considerable time.

In case the frogs are to be preserved immediately after anæsthetizing, an incision should be made through the skin and abdominal wall, a little to one side of the median line, to admit the preserving fluid to the body cavity.

In special cases it may be advantageous to kill the frog by an operation known as "pithing." With the finger-nail locate a notch in the skeleton of the frog on the dorsal side between the skull and the first vertebra. Through this notch make a deep incision with a knife, cutting across the central nervous system. Death is probably instantaneous, but for certain purposes it is desirable to destroy the brain by inserting a needle or a seeker through the incision into the cranial cavity and stirring up the contents. The movements of the limbs of the frog may be quieted by inserting the seeker in the opposite direction and destroying the spinal cord. The method has the advantage of rapidity in case only a few specimens are to be killed, and, if the spinal cord is not destroyed, is especially useful for reflex action experiments; of course it should not be used if the central nervous system is to be dissected.

To preserve the frogs they should always be placed for a time in formalin, which has many advantages over alcohol in preparing material for anatomical work. Formalin does not extract the colors so rapidly as alcohol; it causes a slight swelling of some of the tissues, keeping the specimens in a plump condition, and in particular hardens the muscles without shrinkage; it preserves the central nervous system in a very satisfactory condition for study. Formalin is purchased in a concentrated solution containing 40 per cent. formaldehyde; for practical purposes this is regarded as 100 per cent. formalin. A 2 per cent. solution is strong enough for preserving the frogs, providing the solution is used in sufficient quantity or is changed once after two or three days; a stronger solution may make the specimens too rigid.

After hardening the specimens for a few days or weeks in formalin it is advisable to change them to alcohol for permanent preservation, for the following reasons: (*a*) formalin specimens are unpleasant to handle during dissection since the formalin

vapor is irritating to the eyes, and the solution may attack the fingers causing severe poisoning of the skin; and (*b*) frogs preserved for a long time in formalin are almost worthless for the preparation of skeletons, since the calcareous material of the bones is gradually dissolved, leaving them soft and pliable.

V. Anatomical Preparations.

It is frequently desirable that the student should see a model preparation of a system of organs as a guide for his own dissection, or for the demonstration of features difficult for a beginner to make out. Moreover, as in the case of the skeleton, time and material will be saved by the use of permanent preparations that may be studied by successive classes. At least one example of each of the preparations described in this section should be provided, and it will be profitable to supply as many as the class can use.

Wet preparations should be kept for exhibition in 5 per cent. formalin or 70 per cent. alcohol; in some cases, which will be pointed out, the alcohol has decided advantages. The jars should be of clear glass, tall enough to permit of some evaporation of the liquid before the preparation is exposed. Glass tops, either clamped on over rubber rings or with edges ground and made air tight by smearing with vaseline, are desirable, since metal is attacked by formalin and cork will discolor the alcohol. Each preparation should be provided with an appropriate label.

A. **The Digestive System.**—An entire frog should be prepared with the tongue protruded. This may be accomplished by injecting the lymph space beneath the tongue of a fresh frog with warm gelatin prepared as follows: soak the dry gelatin in distilled water until it is swollen and soft (about 18 hours), then remove to a porcelain evaporating dish and melt at a temperature of about 45° C. The injection causes the tongue to protrude as in the act of seizing food; the pressure should be kept up until the gelatin hardens, when the tongue will be kept permanently in this position. The frog should then be hardened in formalin and exhibited in formalin or in alcohol in a glass jar.

In the study of the digestive system the bile duct and its rela-

tion to the bile sac, pancreas and intestine require special attention. Using a large frog, make a preparation showing the organs represented in figure 42 of Holmes' Biology of the Frog. The parts should be pinned out on a wax plate, such as is used for dissecting trays, which may be made as follows: Melt some beeswax, soft commercial paraffin, or a mixture of these two substances and stir in sufficient lampblack to color it an intense black; pour out into a shallow tray enough of the mixture to form a layer about 8 millimeters thick, and allow it to cool. The plate may then be cut to the required dimensions. The preparation should be exhibited in a rectangular glass jar containing alcohol rather than formalin, since the latter will attack the pins. For fastening the preparation to the wax plate, porcupine quills have some advantages over metallic pins.

B. **The Urogenital System.**—Using fresh material, make preparations of the reproductive systems of a male and a female frog by exposing the organs so far as possible *in situ*. The bladder should be injected with gelatin. In the case of the female, the ovary of one side may be removed in order to display the other organs to better advantage. Since in the female, especially if taken during the breeding season, the oviduct readily absorbs water causing it to swell to an inconvenient size, the use of formalin for hardening and preserving this preparation is not advisable; the dissection should be placed directly in alcohol. A similar preparation should be made of the female reproductive system showing the eggs in the uteri; females in the right condition for this preparation may be found in the early spring.

Of greater importance than the above is the making of permanent preparations showing the urogenital system removed from the body so that all its parts may be separated out clearly and in particular the ducts shown in their relation to the cloaca. The figures in Leuckart's charts on the anatomy of the frog (Kny Scheerer & Co.) will best serve as a guide in making these preparations. Using fresh material, remove the urogenital system entire, together with the large intestine and especially the cloaca, as follows: Dissect the bladder free from the posterior body wall, cut the cloaca across as far back toward the anus as possible, dissect the entire large intestine free from the dorsal body wall,

and cut it across where it is joined by the small intestine. Remove the large intestine, together with the cloaca, bladder, kidneys, gonads, fat bodies and the ducts which enter the cloaca. Spread out the preparation on a wax plate to be used in mounting it (see directions for the digestive system), and clean up the peritoneum and blood vessels about the ureters. Trace the ducts to their union with the cloaca, slit the cloaca open along a ventrolateral side and find the openings of the ducts and the bladder; insert bristles into the openings, using differently colored bristles to distinguish the various kinds of openings. Insert bristles into the openings of the oviducts at their anterior ends. After arranging the various parts in suitable positions so that they may be easily distinguished, fasten them to the wax with pins. In the case of the female urogenital system the preparation must be hardened and preserved in alcohol rather than in formalin.

It often happens that frogs purchased during the winter are nearly all females, hence preparations of the male urogenital system are peculiarly necessary.

C. **The Circulatory System.**—1. The **heart** of the average-sized specimen of Rana pipiens is rather small for satisfactory dissection, consequently this work should be supplemented by the examination of permanent preparations made from the largest frogs obtainable. The preparations should be kept in small cylinder jars or vials, containing alcohol or formalin, in which they may be examined from all sides. The preparations should include some entire hearts with stumps of blood-vessels attached. In particular the structures of the dorsal surface should be shown, since these give most difficulty in the dissection of a small frog; the sinus venosus, venæ cavæ and especially the pulmonary veins should be intact and free from foreign tissue. In addition there should be some dissections of the ventral side showing the internal structures represented in figure 72 of Holmes' Biology of the Frog.

2. The **arteries** should be injected with some colored substance in order that they may be more easily distinguished. For this purpose a starch injection mass is generally used, since it flows freely when injected, and soon hardens so that it will not readily

escape from a vessel accidentally cut. It should be made according to the following formula:

Corn starch	400 parts by volume.
2 per cent. chloral hydrate	400 parts by volume.
95 per cent. alcohol	100 parts by volume.
Color and glycerin (equal parts)	100 parts by volume.

Unless the starch is quite free from lumps it should be ground in a mortar.

The colors most commonly used for the arteries are chrome yellow (lead chromate), vermilion (mercuric sulphid), and red lead. In order to inject the smaller arteries successfully it is necessary that the color should be finely powdered and free from lumps; it is often advisable to grind it thoroughly in a mortar. An extremely fine chrome yellow may be made by dissolving 100 grams lead acetate and 45.5 grams potassium bichromate each in a liter of water. After compete solution, mix and allow the precipitate to settle. Pour off the supernatant fluid, and wash the precipitate in several waters so as to remove the potassium acetate which would injure the specimen. The reaction gives about 100 grams chrome yellow, which should not be allowed to dry before using.

The constituents of the starch mass should be thoroughly mixed by stirring, and the mass strained through muslin, with frequent stirring. The starch and color quickly settle, hence the mixture must be stirred before using, and occasionally while it is being used. It will keep indefinitely.

For injection select the largest frogs, to be used only for the study of the arterial system; they should be killed with ether. Open up the body cavity in the same manner as directed in the text for the study of the viscera, and cut away all that part of the pectoral girdle between the fore-limbs, taking care not to leave any sharp projecting points of bone which might injure the arteries. Cut away the pericardium so as to expose the heart completely.

For injecting, the writer prefers to use a syringe with glass cylinder and metallic frame, of about 10 c.c. capacity; it should be provided with a metallic cannula beveled at the tip so as to present a tapering point. It is important that the cannula should be of

proper caliber to fit accurately into the truncus arteriosus of an average-sized frog; it is well to provide several different sizes, so that a selection may be made. With fine forceps lift up the tip of the ventricle, and with fine scissors make an oblique incision through the wall of the ventricle about midway between its base and apex; through this opening insert the tip of the cannula into the cavity of the ventricle, thence into the truncus arteriosus; some care and skill is required to avoid forcing it into the auricles instead. While making the injection, with forceps hold the parts firmly in place about the cannula; a ligature is unnecessary, since the valves of the truncus arteriosus prevent the escape of the injection mass after the cannula is removed. Inject with slow and steady pressure as much of the injection mass as the arteries will stand without bursting—a point that must be learned by experience.

In case a metallic cannula of the proper size cannot be secured, a cannula may be made by drawing out a piece of glass tubing after heating it in a flame. This may be fastened to the syringe with a piece of rubber tubing, but on account of the flexibility of the connection such a cannula is not so easy to use.

It is advisable to keep the frogs in moderately warm water for a short time immediately before and after the operation. They should be preserved for a few days in weak formalin (2 per cent.), and afterwards changed to 70 per cent. alcohol.

Permanent preparations of some of the best injections may be made, with the principal arteries dissected out, and showing particularly the cœliaco-mesenteric artery and its branches since these often fail of satisfactory injection. Such a preparation may be pinned out on a plate of wax and exhibited in a jar of alcohol.

3. The **veins.** Frogs will be needed especially for the study of the venous system, but the veins need not be injected artificially since the blood affords a natural injection of the veins by collecting in them after death, leaving the arteries empty. The specimens should be killed with ether and preserved in weak formalin (2 per cent. or 3 per cent.); an incision should be made through the ventral wall of the abdomen to allow the formalin to enter the body cavity. They should be rinsed for a few hours in water before being used for dissection.

4. The **capillaries,** and their relation to arteries and veins, are best demonstrated in the web of the foot of a living frog. For this purpose a young frog is best, since the web is thinner and contains less pigment than in an older specimen. Fasten the frog to a small piece of thin board by means of a cloth jacket which will not interfere with the circulation; spread out the toes of a hind foot so that the web will be stretched across a hole near the corner of the board, and tie them in that position with thread. Keep both upper and under surfaces of the web well moistened with normal salt solution, and cover a portion of the web, between two digits, with a triangular piece of cover glass in order that it may be examined with high as well as with low power. The entire frog, excepting the foot, should be covered with a wet cloth. Provide a box of equal height with the stage of the microscope, and place the frog, stretched out on the board, on the box and the stage. Adjust the board so that the web of the frog's foot is in position to be viewed with the microscope; the hole in the board will admit light reflected by the mirror. It may be more convenient to kill the frog (by pithing); it may then be pinned to the board. In such a preparation the circulation will usually continue for several hours.

The capillary circulation may also be demonstrated to good advantage in the mesentery of a frog killed by pithing. Cut through the body wall of one side of the abdomen and pull out a loop of the small intestine. Pin this out on a board in such a manner as to spread out the mesentery and exhibit in the same way as the web of the frog's foot.

D. **The Respiratory System.**—An entire specimen should be prepared with the lungs exposed and inflated. If blown up with air they may be hardened in that condition in formalin.

The vocal sacs of the male may be injected with gelatin and exposed by removing the overlying integument.

Permanent preparations of the hyoid apparatus, larynx and lungs, dissected out as directed in the text, are required.

Separate lungs slightly inflated, then hardened in formalin and cut open so as to expose the interior, should be exhibited in small glass vials.

E. **The Skeleton.**—The following preparations are desirable, the number of each depending on the size of the class:

Complete articulated skeleton, with hyoid intact.
Articulated skull. Disarticulated skull.
Cartilaginous cranium, in alcohol.
Pectoral girdle, in alcohol. Pelvic girdle.
Vertebral column, strung on wire.
Hand and foot, mounted on cardboard.
Hyoid apparatus, in alcohol.
Miscellaneous bones, disarticulated.

Entire articulated skeletons require considerable time for their preparation; good ones may be obtained from dealers at reasonable prices. Articulated skeletons may be prepared from specimens hardened in formalin, provided they have not been preserved too long in the formalin; but the bones must be cleaned by the tedious process of picking away the muscle piecemeal with forceps. Formalin specimens are undesirable for the preparation of disarticulated skeletons, since it is difficult to make even tolerably clean preparations.

Freshly-killed specimens are to be preferred when available, since the task of removing the muscles may then be simplified by boiling. Remove the skin from the frog, separate the forelimbs together with the pectoral girdle, and boil the parts for a few minutes in water, or better, in soap solution prepared by heating and thoroughly mixing the following:

Hard soap	75 grams
Potassium nitrate (saltpeter)	12 grams
Strong ammonia	150 c.c.
Distilled or soft water	2,000 c.c.

For use, one part of this stock solution is diluted with three parts water. If an articulated skeleton is desired, care must be taken not to boil too long, as this will soften and weaken the ligaments. Pick and brush away as much of the flesh as possible, repeating the boiling if necessary.

Complete articulated skeletons are best mounted in glass jars containing alcohol, since in dry preparations the delicate cartilaginous parts such as the hyoid apparatus and the suprascapulas become shriveled. If for any reason a dry preparation is desired, it may be mounted on a wooden base and covered with a rec-

tangular glass case to prevent injury and keep it free from dust. The edges of the glass plates used in making the box may be bound together with passepartout. The best skeletons should be mounted by one or the other of the above methods, but it is desirable to have in addition some articulated skeletons mounted without covering, so that the bones may be demonstrated.

The cartilaginous cranium (see Holmes' Biology of the Frog, figure 65) may be prepared after prolonged boiling or maceration of the skull of a young frog, by carefully picking away the membrane bones.

In making disarticulated preparations of the skull, hands and feet each of these parts should be tied up in cheese cloth so that none of the bones will be lost during prolonged boiling. Each set of bones should be kept in a separate box. In preparing the disarticulated vertebral column it is best to pass a wire through the neural canal before boiling the bones, in order to make sure that the vertebræ are kept in their proper position and order with reference to one another.

The hand and foot should each be mounted by fastening the bones with liquid glue to a piece of stiff black cardboard or "mounting board." The bones should be mounted in their natural position with reference to one another, but slightly separated in order to enable the student to distinguish the different elements. The bones of the wrist and ankle should be included in the preparations of the hand and foot respectively, and in the case of the hand the radio-ulna may well be added. Each preparation should be framed by covering it with a plate of glass of the same size as the cardboard (photographic negatives cleaned by boiling or treating with nitric acid to remove the film are excellent for this purpose), supported by strips of cardboard glued to the margins; the edges of the glass and cardboard may then be bound together with passepartout, used in the same manner as for a lantern slide. The permanency of such preparations will well repay the trouble of making them.

Some of the long bones (*e. g.*, the humerus and the femur) should be prepared by cutting, sawing or grinding them lengthwise so as to exhibit a longitudinal half of the bone with the interior cavity exposed.

F. **The Nervous System.**—Dissections of the central nervous system made by a beginner are seldom satisfactory, hence it is important that a supply of perfect preparations be provided. In particular see that the roots of the spinal and cranial nerves are intact. For these preparations choose large frogs which should be well hardened in strong formalin (10 per cent.) before the dissection is attempted; it is well to remove the roof of the cranium in order to admit the formalin. These preparations are best kept in glass vials so that they may be viewed from all sides. Permanent preparations showing the spinal nerves and the sympathetic nervous system in situ are desirable.

VI. Histological Preparations.

The making of permanent histological preparations should hardly be attempted by a person without experience, except under competent guidance. For general methods of procedure in mounting whole objects and preparing serial sections, as well as for some special methods applicable to the frog, see Guyer, Animal Micrology (University of Chicago Press). The following hints may prove helpful.

Most of the material to be sectioned may be very satisfactorily fixed in Zenker's fluid, which should be used in abundance for from 6 to 24 hours depending on the size of the object. Wash in running water, or in frequent changes of water, for from 12 to 24 hours; to insure thorough washing it is well to keep the object for several days in weak formalin (3 per cent. to 5 per cent.), changing the formalin as often as it becomes discolored, until the object changes from a yellow color to drab. The washing should be done in the dark (cover the material with a black cloth). It is important to remember that in all cases of fixation in a solution containing corrosive sublimate, the material must be treated with 70 per cent. alcohol containing iodin, in order to remove the crystals.

For staining sections, two general methods are available: (*a*) The sections may be stained on the slide with a nuclear stain (*e. g.,* Delafield's hæmotoxylin), and counterstained on the slide wtih a cytoplasmic stain (*e. g.,* Orange G or eosin). Orange G is to be preferred to eosin since the latter may fade in time, but

the sections must be stained deeply with the Orange G, and the slides passed very rapidly to the higher alcohols, otherwise this stain will wash out in the lower alcohols. Staining on the slide with Delafield's hæmotoxylin and Orange G is a time-consuming process in case a large number of slides are to be prepared, but the practical certainty of getting good results, and the permanency of the stains, make this method decidedly to be preferred for most tissues of the frog. (*b*) A more rapid method, so far as the actual work is concerned, is to stain the material in toto in Grenacher's borax carmine for a day or two, and destain for several hours, to remove the stain from the cytoplasm, in acid alcohol (0.25 per cent. hydrochloric acid in 70 per cent. alcohol). Be careful not to destain too long; the process should be stopped as soon as the object changes from a dull red to a slightly pinkish hue. Counterstain on the slide with Lyons blue to which sufficient picric acid has been added to change it to green. Be careful not to counterstain too deeply, and wash in at least one change of xylol. The counterstain gives remarkably good differentiation of the cytoplasmic structures, but it will fade in time. In order to stain the nuclei with sufficient intensity in the borax carmine, be careful to get a good preparation of this stain, and if necessary stain for two days, keeping the material in a warm place. The object should have an acid reaction. Certain objects (*e. g.*, the stomach, intestine and kidney) after fixation in Zenker's are sometimes very resistent to this stain; in such cases Mayer's paracarmine may be substituted for borax carmine and the object washed for an hour or two in 1 per cent. acetic acid.

As a general rule the paraffin method of imbedding and cutting is to be preferred. Paraffin with a melting-point of about 52° C. is best for general use. Thin sections must be cut at a low room temperature, thick sections at a high room temperature. The sections are best cut with a Minot rotary microtome. Xylol is usually to be preferred as a clearing reagent, and Canada balsam for the final mounts.

The following preparations are needed: 1. **Stratum corneum.** Shed epidermis (stratum corneum) may be found in the water in which living frogs are kept; this is usually satisfactory for study when preserved promptly in formalin. The material should

be collected when available, and a supply kept on hand; it furnishes a good object with which to begin the study of the tissues. Permanent stained mounts are hardly necessary; if they are desired, the material should be treated with corrosive-acetic, stained with a nuclear stain such as Delafield's hæmotoxylin, and counterstained with Orange G or with eosin.

2. **Cartilage.** Cartilage taken from frogs hardened in formalin in the usual way is seldom in good condition, since the formalin does not penetrate quickly enough. Material for this study should be prepared by placing the fresh femur, or other parts of the skeleton containing hyaline cartilage, in strong formalin (10 per cent.); or the fresh cartilage may be examined in normal salt solution (for formula see foot-note, p. 45). Free-hand sections should be cut with a razor. Such preparations are likely to be more satisfactory than permanent stained mounts.

3. **Blood.** Permanent preparations are desirable to show more clearly the nuclei of the red blood corpuscles. A rapid method is is to smear a thin film of blood on a slide, fix it in a saturated solution of corrosive sublimate, and stain in Delafield's hæmotoxylin, counterstaining with Orange G or with eosin. For details of this and other methods see Guyer, Animal Micrology.

4. **Non-striated muscle fiber.** After ligaturing the intestine of a freshly-killed frog, inflate the bladder with air and place it in the fixing fluid (corrosive-acetic or Zenker's) while still inflated; or stretch it out on a piece of cork and submerge in the fixing fluid. Stain with Delafield's hæmotoxylin, and counterstain with Orange G or with eosin. Cut it into pieces of suitable size for mounting.

5. **Connective tissue.** As directed in the text, material may be taken from a frog hardened in formalin, and studied without staining, but there are some advantages in using permanent stained preparations for comparison. The thin septa which fasten the skin of the frog to the body consist for the most part of white fibrous connective tissue; cut out pieces of this from a fresh frog, spread on a slide and fix with corrosive sublimate (avoid the use of fixing fluids containing acetic acid, since this tends to dissolve the white fibers leaving the yellow elastic fibers exposed). Stain with Mallory's connective tissue stain (see Guyer, Animal Micrology).

6. **Cross-sections of the intestine.** Using a freshly-killed frog that has not taken food for several days, cut out short pieces of the intestine (about 1 centimeter long) with a narrow strip of the mesentery attached. Fix 12 to 16 hours in Zenker's fluid. Stain on the slide with Delafield's hæmotoxylin and counterstain with Orange G; or stain in bulk with borax carmine or paracarmine and counterstain with the Lyons-blue picric-acid mixture. The latter method gives better differentiation of the goblet cells. Cut sections 6μ to 8μ thick.

7. **Cross-sections of the stomach.** Using a freshly-killed young frog with stomach in the resting condition, cut out the entire stomach and fix 24 hours in Zenker's fluid (corrosive-acetic does not penetrate well enough to fix the nuclei of the mucosa satisfactorily). After passing the object to 96 per cent. alcohol, trim off the pyloric portion and the extreme cardiac end. Stain by either method recommended for the intestine. Sections should be cut with a thickness not exceeding 8μ.

8. **Cross-sections of the kidney.** Remove the kidney from a fresh frog, cutting around the organ so as to include the peritoneum and adjacent portion of the ureter (care must be taken not to tear off the peritoneum from the dorsal surface of the kidney). Fix about 12 to 16 hours in Zenker's fluid. Discard the anterior third and the extreme posterior end, and cut sections 8μ in thickness. Stain on the slide with Delafield's hæmotoxylin and Orange G.

9. **Sections of the thyroid gland.** Since the gland is extremely small, a piece of cartilage may be left attached to aid in handling it. Fix for a few hours in Zenker's, cut thin sections and stain with Delafield's hæmotoxylin and Orange G.

10. **Cross-sections of the integument.** Select a frog whose skin is bright-colored, and kill it by pithing, not with anæsthetics since the latter may cause too profuse secretion from the mucus and poison glands. From the dorsal surface cut out a strip that includes both lightly-pigmented and deeply-pigmented portions as well as some poison glands (the latter are especially numerous in the dorso-lateral line). Fix for a few hours in Zenker's; stain on the slide with Delafield's hæmotoxylin and Orange G, or stain in bulk with borax-carmine and counterstain

on the slide rather deeply with the Lyons-blue picric-acid mixture. Cut sections 8 μ thick.

11. **Cross-section of decalcified femur.** Cut out a piece about 1 centimeter long from the middle of the femur, and fix for a week or two in Müller's fluid; decalcify for several weeks in 70 per cent. alcohol containing 10 per cent. nitric acid. Stain with borax carmine; a counterstain is not required. The sections may be cut in paraffin if the decalcification is thorough.

12. **Cross-sections of mammalian bone, dry.** With a fine saw cut a thin transverse section of the femur of a cat, rabbit, pig or other small mammal. Let it macerate in water until quite clean, then dry it thoroughly. Cement the disc of bone to a plate of glass by means of Canada balsam; grind it to the required thinness on a clean hone. The section is not thin enough until fine print can be distinguished through it. Remove the section by immersing in xylol, remove the xylol with absolute alcohol, wash the section thoroughly in water, transfer it to absolute alcohol for ten minutes, then to pure ether for half an hour. Clamp the section between two slides by means of rubber bands, and allow it to dry thoroughly. Break the section into pieces of suitable size for mounting. Mount dry, fastening the edges of the cover glass with cement; or place some xylol-balsam in the center of the slide and heat it for a few minutes to drive off the xylol, then press the section of bone firmly into it and put on a cover glass. The balsam should not be thin enough to penetrate the tissue of the bone.

13. **Cross-sections of the spinal cord.** Be careful to leave the investing membranes intact. Fix in Zenker's fluid. Stain in bulk with borax carmine and counterstain on the slide with the Lyons-blue picric-acid mixture; or stain on the slide with Delafield's hæmotoxylin and Orange G. Cut sections 8 μ to 10 μ.

14. **Cross-sections of the brain.** Be careful to leave the investing membranes intact. Fix in Zenker's fluid. Since the sections are to be used for anatomical rather than for histological purposes, the method of staining is not important; the borax-carmine Lyons-blue picric-acid method is to be preferred on account of its rapidity. Cut rather thick sections (10 μ to 15 μ).

15. **Cross-sections of the auditory region.** Use the head of a

very young frog or toad, recently metamorphosed. Fix in Müller's or Tellyesnicky's fluids. Decalcify with 70 per cent. alcohol containing 10 per cent. nitric acid. Stain in bulk with borax carmine or with paracarmine. In cutting the sections be careful to orient the object so as to secure exact cross-sections, some of which will pass longitudinally through the columella. Cut rather thick sections (about 15 μ).

16. **Cross-sections of entire frogs.** Obtain some very small frogs or toads recently metamorphosed, fix in Tellyesnicky's fluid, decalcify, stain in toto with borax carmine or with paracarmine and cut the entire body into thick transverse sections (15 μ to 20 μ). As a review exercise have students sketch sections taken at random, and label the parts.

LABORATORY GUIDE

FOR THE STUDY OF

THE FROG

AN INTRODUCTION TO ANATOMY, HISTOLOGY AND PHYSIOLOGY

BY

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